TRADE SECRET

STUDY TITLE: H-28548: Toxicokinetic Study in Pregnant Rats

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ORIGINAL REPORT COMPLETED: March 29, 2011

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PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-18405-849

WORK REQUEST NUMBER: 18405

SERVICE CODE NUMBER: 849

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: _____ 11 April 2011
Susan M. Munley, M.A. — Date
Research Toxicologist

Sponsor: _____ _____
Sponsor Representative — Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 18405
Service Code Number: 849

Key inspections for the above referenced study were completed by the Quality Assurance Unit of DuPont Haskell and the findings were submitted on the following dates:

Audit Dates	*Date Reported to Principal Investigator*	*Date Reported to PI Management*	*Date Reported to Study Director*	*Date Reported to SD Management*
Protocol:				
June 04, 2010			June 04, 2010	June 04, 2010
Conduct:				
June 14, 2010			June 14, 2010	June 14, 2010
July 07, 2010			July 07, 2010	July 07, 2010
Report/Records:				
August 05,06, 2010	August 06, 2010	August 06, 2010	August 06, 2010	August 06, 2010
August 18-20,23, 2010			August 23, 2010	August 23, 2010
October 01, 04, 2010			October 04, 2010	November 01, 2010
October 28, 29, 2010			October 29, 2010	March 07, 2011
February 25, 2011			February 25, 2011	February 25, 2011
Report Revision 1:				
April 6, 2011	April 6, 2011	April 6, 2011	April 6, 2011	April 6, 2011

Reported by: Donna M. Johnston for A.P. 07 April 2011
Antonio Pedulla Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Dose Formulation Analytical Evaluation by: _____ 08-Apr-2011
Z. Amanda Shen, Ph.D. — Date
Senior Research Chemist

Toxicokinetic Evaluation by: _____ 07-April-2011
Shawn A. Gannon, B.S. — Date
Senior Staff Toxicologist

Toxicokinetic Sample Analysis by: _____ 08-Apr-2011
Michael P. Mawn, Ph.D. — Date
Senior Research Chemist

Approved by: _____ 11-Apr-2011
Scott E. Loveless, Ph.D. — Date
Manager

Reviewed by: _____ 11-Apr-2011
Joseph M. Lewis, B.A. — Date
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Issued by Study Director: _____ 11-April-2011
Susan M. Munley, M.A. — Date
Research Toxicologist

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STUDY INFORMATION

Substance Tested:	• HFPO Dimer Acid Ammonium Salt • 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt • 62037-80-3 (CAS Number) • H-28548
Haskell Number:	28548
Composition:	Proprietary
Purity:	84%
Physical Characteristics:	Clear and colorless liquid
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Study Initiated/Completed:	June 4, 2010 / (see report cover page)
Experimental Start/Termination:	June 7, 2010 / July 8, 2010
In-Life Initiated/Completed:	June 7, 2010 / June 21, 2010
Notebook Number(s):	E-113111-BZ

REASON FOR REVISION 1

To correct an inadvertent typographical error in the sentence that compared the maternal plasma concentration values on days 6 and 20. In the original sentence, the references to days 6 and 20 were reversed. This revision corrects this error. The affected sentences appear on pages 9, 18, 102, and 106 of the original report and are corrected on pages 9, 19, 103, and 107 of the current revised report.

SUMMARY

Groups of presumed pregnant Crl:CD(SD) rats (5 per group) were administered solutions of the test substance, H-28548, in deionized water once daily via oral gavage on gestation days 6 through 20. The dose levels tested were 0, 5, 10, 100, and 1000 mg/kg/day, and doses were administered at a volume of 10 mL/kg. The dose formulations were analyzed and confirmed to be at target concentrations and stable under the conditions of the experiment.

Blood was collected from all animals at euthanasia on gestation day 20 approximately 2 hours following the last dose. Trunk blood was collected from all fetuses and pooled by litter. An additional group of rats at 1000 mg/kg/day was bled via the tail vein on gestation day 6, as well as at the terminal blood collection time point. This group was added to provide toxicokinetic data following a single dose, as compared with after 15 consecutive doses on gestation day 20.

The measured plasma concentrations are summarized below:

Summary of Plasma Concentrations for Parent Compound H-28548

		Concentration (ng/mL)						
		Dams				Pooled Pups		
	Dose	Day 6		Day 20		Day 20		Pup:Dam
Group	(mg/kg/day)	Mean	SD	Mean	SD	Mean	SD	Plasma Ratio
1	0			33	16	19	23	
2	5			3984	469	1134	175	0.3
3	10			9312	1710	2458	465	0.3
4	100			85560	10092	18320	9128	0.2
5	1000			338400	160168	99800	26482	0.3
6	1000	430600	162712	348400	130362	102240	28295	0.3

The toxicokinetic data indicated that the dose response curve was linear between 5 and 100 mg/kg/day. At 1000 mg/kg/day the concentration was less than what would be predicted if the dose response curve was linear through 1000 mg/kg/day.

The mean plasma concentration on day 20 was less than the mean plasma concentration on day 6. This implies that steady state was achieved by day 6 and that there is no accumulation in the dams between day 6 and day 20.

The concentration in plasma pooled from pups was approximately one-third of the concentration in plasma from the dam at the same time point.

OBJECTIVE

The objective of this study was to measure levels of the test substance, H-28548, in plasma prepared from blood collected from pregnant rats on gestation day (GD) 20 and from GD 20 fetuses. Plasma samples from pregnant rats at the highest dose level of 1000 mg/kg/day on GD 6 were also collected and analyzed.

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for DuPont Haskell adhere to the following principles:

- The sponsor and/or the study director ensure that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.

- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.

- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee. The sponsor was advised by the study director of all circumstances that could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.

- Animals were provided with species-appropriate environmental enrichment.

- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

STUDY DESIGN

A. Treatment Groups and Daily Dosage

Group	Dosage (mg/kg/day)[a]	Formulation Concentration (mg/mL)[b]	Number of Timed Mated Females
1	0[c]	0	5
2	5	0.5	5
3	10	1	5
4	100	10	5
5	1000	100	5
6	1000	100	5

a Formulations of test substance in deionized water were administered once daily by gavage on GD 6-20 at a dosing volume of 10 mL/kg.
b To achieve these concentrations of active ingredient, the formulations were adjusted for sample purity.
c The control group animals received vehicle, deionized water, only at a dosing volume of 10 mL/kg.

B. Dosing and Sacrifice Schedule

Rats were dosed once daily by oral gavage on GD 6 to 20 at the dose levels listed in the table above. The volume administered (10 mL/kg) was based on the most recent body weight.

Rats assigned to group 6 were bled via tail vein on GD 6 two hours (± 5 minutes) following dosing.

Rats assigned to groups 1 through 6 were euthanized on GD 20 two hours (± 5 minutes) following dosing; blood was collected at sacrifice and selected tissues were weighed (livers, kidneys) and retained (livers, kidneys, ovaries, uterus).

A maximal volume of blood was collected from each rat at sacrifice and split for preparation of plasma and serum samples. In addition, trunk blood from fetuses was also collected and pooled by litter for preparation of plasma samples.

C. Selection of Route of Administration and Dose Levels

The test substance formulations were administered orally because previous developmental and reproductive toxicity studies[1,2,3] were conducted using the same route of exposure.

The doses selected for the current study are 0, 5, 10, 100, and 1000 mg/kg/day. These doses have been tested in the previously cited studies and the current study was intended to provide information regarding the relative internal doses relevant to the previous work.

MATERIALS AND METHODS

A. Vehicle

The vehicle was deionized water. There were no known contaminants in the vehicle that would be expected to have had any adverse impact on the integrity of the study. The vehicle was assumed to be stable under the conditions of the study. The vehicle was stored at room temperature.

B. Test Substance

(Appendix A)

The test substance, H-28548, was supplied by the sponsor as a clear and colorless liquid with a purity of 84%. The test batch used for this study was assigned Haskell number 28548.

C. Dosing Formulations

1. Preparation

Formulations of the test substance in the vehicle were prepared and used within the period of established stability. Formulation stability was established previously in a separate study[(4)] and demonstrated that the test substance formulations were stable at room temperature for up to 12 days at concentrations ranging from 0.01 to 100 mg/mL. Dosing formulations were stored at room temperature until used. The method of mixing the test substance with the vehicle was documented in the study records.

2. Sampling and Analysis

(Appendix B)

Samples of each formulation were taken 2 times; near the beginning and end of the study. Analyses addressed concentration verification

Samples were analyzed by the DuPont Haskell Analytical Chemistry Group on the day the samples were collected. The Analytical Method used was documented in the study records and is included in this report in Appendix B.

D. Test System

Species/Strain:	Crl:CD(SD) rat
Sex:	Female (nulliparous, timed mated, GD 0 = day mating confirmed)
Supplier:	Charles River Laboratories, Inc., Raleigh, North Carolina
Gestation Day at Arrival:	GD 2
Number Received:	30 received on June 3, 2010

Age at Arrival:	Approximately 86 days
Age at Start of Study:	Approximately 91 days
Weight at Arrival:	Approximately 225-253 grams
Identification:	Each animal was identified by the supplier prior to shipping by marking the tail in indelible ink with a unique number from a list provided by DuPont Haskell. The tail marks were verified routinely and darkened, if necessary, to ensure tracking of animal identity.

GD 0 body weights were supplied by the vendor and included in the study records.

E. Justification for Animal Model

The rat was selected for this study because it is a preferred species for developmental toxicity testing as recommended by test guidelines. The Crl:CD(SD) strain was chosen because extensive background information is available from the literature, the supplier, and previous studies conducted at DuPont Haskell. This strain is also considered suitable relative to hardiness and incidence of spontaneous disease.

F. Animal Husbandry

Housing:	individually in solid bottom caging with bedding, and nestlets as enrichment
Cage Rack Positioning:	Cage racks were not relocated within the animal room.
Climate:	Temperature of 18-26°C (64-79ºF) Relative humidity of 30%-70% Any excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study. The relative humidity and temperature values in the housing rooms were monitored continuously and recorded automatically at regular intervals each day. The temperature and humidity records were reviewed by the laboratory veterinarian and/or designee; a copy of the animal room accountability records, which contain manually recorded temperature and humidity checks (once or twice daily during study), are retained with the study records.
Illumination:	Artificial (fluorescent light) on an approximate 12-hour light/dark cycle.
Water:	Tap water *ad libitum*
Feed:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 (pellets) *ad libitum*.

1. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.

- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

G. Quarantine and Pretest Period

Rats were quarantined for at least 3 days and then released for the study based on acceptable body weights and freedom from any adverse clinical observations.

H. Assignment of Animals to Groups

Before dosing began, animals were randomly assigned to control or experimental groups using a computerized randomization procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot.

I. In-life Observations

Procedure	Frequency
Quarantine and Pretest	
Mortality/Moribundity	At least once daily
Clinical Observations	GD 4
Body Weights	GD 4
Food Consumption	GD 4

Procedure	Frequency
Testing Period	
Mortality/Moribundity	Twice daily (AM and PM)
Blood Collection	For animals in group 6, once 2 hours (± 5 minutes) post-dosing on GD 6
Clinical Observations	Twice daily on GD 6-19 (during weighing and at least 2 hours post-dosing) Once on GD 20
Body Weights	Daily on GD 6-20
Food Consumption	GD 6, 8, 10, 12, 14, 16, 18, and 20

J. Animal Euthanasia

Adult females were euthanized by carbon dioxide asphyxiation and exsanguination; a maximal volume of blood was collected. The whole blood was divided so that both plasma and serum could be prepared.

Fetuses were euthanized by decapitation and trunk blood was collected and pooled by litter. Plasma was prepared from the whole blood samples collected for each litter.

K. Postmortem Evaluations

1. Females Surviving to Scheduled Euthanasia

Gross external and a visceral examinations were performed immediately after euthanasia.

The uterine horns from uteri with no visible implantation sites were placed in a 10% aqueous solution of ammonium sulfide[5] to detect very early resorptions. The uterine body was frozen for possible future evaluation.

For each animal, blood was collected and prepared to plasma and serum. In addition, the weight of the liver and kidneys were recorded. For each female, kidneys, a portion of the liver, and one ovary were placed in formalin. The remainder of the liver, uterus, and the remaining ovary were flash frozen in liquid nitrogen and stored between -60ºC and -80ºC. These tissues were retained for possible future histopathologic examination.

For each female with visible implantation sites, the types of implantations (live and dead fetuses, early and late resorptions) and their relative positions in the uterus were recorded. Live fetuses were decapitated to collect trunk blood which was pooled by litter and prepared to plasma.

The types of implantations were classified as follows:

live fetus:	fully formed and responds to stimuli
dead fetus:	fully formed with little or no evidence of maceration
late resorption:	identifiable structures (i.e. digital rays)
early resorption:	no visible fetal structures

L. Blood Collection and Plasma Analysis

Whole blood was collected from rats via tail vein in Group 6 two hours (± 5 minutes) after dosing on GD 6. The actual time of blood collection was recorded in the study records. Whole blood from the animals in group 6 as well as from all other groups 1 through 5 was collected from the *vena cava* at sacrifice. Whole blood from fetuses was collected from the trunk following decapitation and pooled by litter.

Whole blood samples were collected using EDTA or heparin as an anticoagulant and processed to plasma by centrifugation. The red blood cell fraction was discarded after separation. Plasma was stored frozen at ≤ 20ºC until delivery to the DuPont Haskell Analytical Chemistry Group.

Samples were analyzed for the test substance using LC-MS-MS. The analytical method used can be found in Appendix K.

M. Serum Preparation and Storage

A minimum of 0.6 mL of whole blood was placed in a serum separator tube on ice until the serum was prepared. Serum was stored between -60ºC and -80ºC for possible future analysis.

N. Ovary/Uterus Collection and Storage

For each rat, one ovary and the uterus were collected and flash frozen in liquid nitrogen and stored between -60ºC and -80ºC for possible future analysis.

STATISTICAL ANALYSES

Descriptive statistics were performed on endpoints evaluated for this study.

RESULTS AND DISCUSSION

Formulation Analytical Evaluation

A. Dosing Formulations Analysis

(Appendix C)

Data from the analysis of the formulation samples indicated that the test substance was at the targeted concentrations for the study. Test substance was not found in the 0 mg/mL samples (control).

In-Life, Maternal Gross, and Caesarian Section Data

Data collected during the in-life period of the study were collected for the purpose of monitoring the health of the animals and for calculating individual dose volumes.

A. Maternal Mortality and Clinical Observations

(Table 1, Appendix D)

There was no test substance-related mortality at any level tested; all animals on study survived until scheduled euthanasia.

There were no test substance-related clinical observations at any level tested; the observations that were recorded were unremarkable and occurred infrequently.

B. Maternal Body Weights, Body Weight Changes, Food Consumption

(Tables 2-4, Appendices E-G)

Data for body weights, weight changes, and food consumption were collected for the purpose of monitoring the health of the animals and for calculating the daily dose volumes. Evaluation for potential dose-related effects evident in these endpoints in pregnant rats at the same dose levels has previously been conducted in the developmental toxicity study with the test substance (18405-841). Therefore, these data are provided in the current report primarily to confirm the health status of the animals.

C. Maternal Gross Postmortem Findings

(Table 5, Appendix H)

All females appeared grossly normal at necropsy with the exception of a single adult female at 1000 mg/kg/day that had liver discoloration.

D. Reproductive Outcome and Litter Data

(Table 6, Appendix I)

All females were pregnant with the exception of a single control group female. Data for litter sizes and incidences of resorptions were comparable across all groups tested.

E. Maternal Liver and Kidney Weights

(Table 7, Appendix J)

Mean liver weights were 12 to 18% higher than controls at 100 and 1000 mg/kg/day. Otherwise, mean liver weights at the lower levels and mean kidney weights at all dose levels were generally comparable.

Toxicokinetics Conclusions

A. Plasma Analysis

(Appendix K)

The measured plasma concentrations for dams and fetuses are provided in Text Table 1:

Text Table 1: Summary of Plasma Concentrations for Parent Compound H-28548

		Concentration (ng/mL)						
		Dams				Pooled Pups		
	Dose	Day 6		Day 20		Day 20		Pup:Dam
Group	(mg/kg/day)	Mean	SD	Mean	SD	Mean	SD	Plasma Ratio
1	0			33	16	19	23	
2	5			3984	469	1134	175	0.3
3	10			9312	1710	2458	465	0.3
4	100			85560	10092	18320	9128	0.2
5	1000			338400	160168	99800	26482	0.3
6	1000	430600	162712	348400	130362	102240	28295	0.3

The dose response curve was linear between 5 and 100 mg/kg/day. At 1000 mg/kg/day the concentration was less than what would be predicted if the dose response curve was linear through 1000 mg/kg/day.

The mean plasma concentration on day 20 was less than the mean plasma concentration on day 6. This implies that steady state was achieved by day 6 and that there is no accumulation in the dams between day 6 and day 20.

The concentration in plasma pooled from pups was approximately one-third of the concentration in plasma from the dam at the same time point.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

REFERENCES

1. WIL Research Laboratories, LLC (in progress). An Oral (Gavage) Prenatal Developmental Toxicity Study of H-28548 in Rats. Unpublished report, DuPont-18405-841.

2. DuPont Haskell (in progress). H-28548: Follow-Up Developmental Toxicity Study in Rats. Unpublished report, DuPont-18405-840.

3. WIL Research Laboratories, LLC (in progress). An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice. Unpublished report, DuPont-18405-1037.

4. WIL Research Laboratories, LLC (in progress). A 90-Day Oral (Gavage) Study of H-28548 in Rats with a 28-Day Recovery. Unpublished report, WIL-189216.

5. Salewski, E. (1964). Farbemethode zum makroskopischen Nachweis von Implantationstellen am Uterus der Ratte. *Archiv. Path. Exp. Pharmakol.* **247**, 367.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

-/---/./…/Blank space	-	no data/data could not be calculated
kg	-	milogram
mg	-	milligram
N/n	-	number in group/number of values used in calculation of mean
Ma	-	mating

NOTES:

Due to rounding differences, values in tables may be slightly different than appendices.

Table 1
Summary of Maternal Clinical Observations

Day numbers relative to Mating Date

Sex: Female

	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
Scheduled sacrifice						
Number of Observations	5	5	5	5	5	5
Number of Animals	5	5	5	5	5	5
Days from - to	20 20	20 20	20 20	20 20	20 20	20 20
Hair loss						
Number of Observations	12	.	.	.	.	.
Number of Animals	1	.	.	.	.	.
Days from - to	9 20	.	.	.	.	.
Mass						
Number of Observations	.	.	6	.	.	.
Number of Animals	.	.	1	.	.	.
Days from - to	.	.	15 20	.	.	.
Wet fur						
Number of Observations	.	.	.	1	7	.
Number of Animals	.	.	.	1	2	.
Days from - to	.	.	.	14 14	8 17	.

Table 2
Mean Maternal Body Weights

Bodyweight (g)

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
6	Mean	271.8	267.6	264.7	275.8	270.6	268.0
	SD	15.7	10.4	16.8	9.4	21.0	16.7
	N	5	5	5	5	5	5
	%Diff	-	-1.6	-2.6	1.5	-0.4	-1.4
7	Mean	275.3	271.1	266.0	281.5	269.1	260.0
	SD	18.3	8.6	20.3	9.0	17.6	19.5
	N	5	5	5	5	5	5
	%Diff	-	-1.5	-3.4	2.3	-2.2	-5.5
8	Mean	276.8	275.2	266.6	282.9	270.6	265.8
	SD	19.2	8.8	21.5	6.6	18.1	18.5
	N	5	5	5	5	5	5
	%Diff	-	-0.6	-3.7	2.2	-2.2	-4
9	Mean	275.2	280.1	270.8	288.4	271.6	267.4
	SD	25.3	10.8	22.7	7.2	19.1	19.4
	N	5	5	5	5	5	5
	%Diff	-	1.8	-1.6	4.8	-1.3	-2.8
10	Mean	279.1	284.7	281.0	298.8	277.3	274.9
	SD	28.4	9.3	20.8	8.5	21.3	19.4
	N	5	5	5	5	5	5
	%Diff	-	2	0.7	7.1	-0.6	-1.5
11	Mean	278.5	297.3	290.8	306.8	287.1	281.3
	SD	35.1	10.2	20.3	9.7	21.5	19.5
	N	5	5	5	5	5	5
	%Diff	-	6.7	4.4	10.1	3.1	1

Statistical Test: Anova & Dunnett's Test Transformation: Automatic

Table 2
Mean Maternal Body Weights (Continued)

Bodyweight (g)

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
12	Mean	299.6	303.1	297.8	310.2	291.8	285.9
	SD	21.1	9.3	23.5	11.5	20.0	18.0
	N	5	5	5	5	5	5
	%Diff	-	1.1	-0.6	3.5	-2.6	-4.6
13	Mean	302.7	303.9	302.0	317.7	296.5	289.8
	SD	23.6	7.9	24.0	11.1	19.0	18.8
	N	5	5	5	5	5	5
	%Diff	-	0.4	-0.2	5	-2	-4.3
14	Mean	308.8	308.2	304.3	320.7	302.5	295.0
	SD	24.4	7.6	27.0	9.2	22.7	17.8
	N	5	5	5	5	5	5
	%Diff	-	-0.2	-1.5	3.9	-2	-4.4
15	Mean	315.3	313.0	311.6	329.2	309.9	301.0
	SD	23.5	6.6	26.4	10.6	19.8	20.2
	N	5	5	5	5	5	5
	%Diff	-	-0.7	-1.2	4.4	-1.7	-4.5
16	Mean	321.1	318.2	320.4	339.1	318.4	310.9
	SD	26.4	7.8	27.3	14.1	18.4	18.1
	N	5	5	5	5	5	5
	%Diff	-	-0.9	-0.2	5.6	-0.8	-3.2
17	Mean	338.4	333.4	334.3	353.0	336.8	323.6
	SD	25.6	16.2	32.5	10.1	20.2	20.1
	N	5	5	5	5	5	5
	%Diff	-	-1.5	-1.2	4.3	-0.5	-4.4

Statistical Test: Anova & Dunnett's Test Transformation: Automatic

Table 2
Mean Maternal Body Weights (Continued)

Bodyweight (g)

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
18	Mean	352.6	343.7	350.6	371.0	355.8	342.5
	SD	26.4	22.0	28.6	8.5	15.5	19.5
	N	5	5	5	5	5	5
	%Diff	-	-2.5	-0.6	5.2	0.9	-2.9
19	Mean	368.6	360.9	365.8	385.1	367.4	357.8
	SD	23.9	17.7	29.4	11.1	17.1	20.2
	N	5	5	5	5	5	5
	%Diff	-	-2.1	-0.7	4.5	-0.3	-2.9
20	Mean	379.6	376.8	377.8	397.4	371.6	362.1
	SD	27.9	17.1	29.0	11.5	12.2	19.7
	N	5	5	5	5	5	5
	%Diff	-	-0.7	-0.5	4.7	-2.1	-4.6

Statistical Test: Anova & Dunnett's Test Transformation: Automatic

Table 3
Mean Maternal Body Weight Gains

Body Weight Gain

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
6 → 8	Mean	5.0	7.6	1.9	7.1	0.0	-2.1
	SD	5.6	3.4	7.3	4.4	6.8	3.1
	N	5	5	5	5	5	5
	%Diff	.	52.6	-62.7	43.4	-100.8	-142.6
8 → 10	Mean	2.3	9.5	14.4	15.9	6.7	9.0
	SD	17.2	4.7	7.4	4.3	7.4	1.5
	N	5	5	5	5	5	5
	%Diff	.	318.4	532.5	596.5	194.7	296.5
10 → 12	Mean	20.6	18.4	16.8	11.4	14.5	11.0
	SD	11.9	5.7	4.0	5.6	6.5	3.7
	N	5	5	5	5	5	5
	%Diff	.	-10.7	-18.3	-44.6	-29.6	-46.5
12 → 14	Mean	9.1	5.1	6.5	10.5	10.7	9.2
	SD	3.9	2.7	7.1	4.3	4.6	3.5
	N	5	5	5	5	5	5
	%Diff	.	-43.8	-28.9	15.1	17.5	0.2
14 → 16	Mean	12.4	10.0	16.1	18.4	15.9	15.9
	SD	5.7	4.6	3.5	5.0	5.5	5.7
	N	5	5	5	5	5	5
	%Diff	.	-19.4	30.3	48.5	28.8	28.5
16 → 18	Mean	31.5	25.5	30.3	31.9	37.3	31.6
	SD	4.6	16.4	2.6	6.1	4.1	5.6
	N	5	5	5	5	5	5
	%Diff	.	-18.8	-3.8	1.5	18.7	0.3

Statistical Test: Anova & Dunnett's Test Transformation: Automatic

Table 3
Mean Maternal Body Weight Gains (Continued)

Body Weight Gain

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
18 → 20	Mean	27.0	33.1	27.2	26.4	15.8	19.7
	SD	5.4	10.0	2.3	3.6	8.9	7.2
	N	5	5	5	5	5	5
	%Diff	.	22.5	0.8	-2	-41.4	-27.1

Statistical Test: Anova & Dunnett's Test Transformation: Automatic

Table 4
Mean Maternal Food Consumption

Food Consumption Mean

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
6 → 8	Mean	21.2	20.5	18.9	23.4	17.1	14.7
	SD	3.8	1.6	4.5	1.4	2.8	3.1
	N	5	5	5	5	5	5
	%Diff	.	-3.4	-11.1	10	-19.7	-30.6
8 → 10	Mean	20.0	21.1	21.4	23.7	18.2	18.2
	SD	4.4	1.7	3.4	3.1	3.4	2.8
	N	5	5	5	5	5	5
	%Diff	.	5.5	6.8	18.3	-8.9	-9.2
10 → 12	Mean	22.5	23.9	25.3	25.8	22.6	19.5
	SD	4.4	1.0	3.6	1.7	3.0	3.0
	N	5	5	5	5	5	5
	%Diff	.	6.4	12.6	14.7	0.7	-13.2
12 → 14	Mean	24.7	22.4	24.3	25.9	25.0	21.4
	SD	4.4	1.4	4.2	0.9	2.9	2.1
	N	5	5	5	5	5	5
	%Diff	.	-9.4	-1.6	4.8	1.1	-13.7
14 → 16	Mean	24.9	22.4	23.9	26.5	25.5	22.5
	SD	3.2	0.9	3.7	1.9	1.4	3.6
	N	5	5	5	5	5	5
	%Diff	.	-10.1	-3.7	6.4	2.6	-9.4
16 → 18	Mean	27.7	22.7	27.3	28.0	28.2	24.3
	SD	3.6	7.2	3.9	1.5	2.6	3.4
	N	5	5	5	5	5	5
	%Diff	.	-18.2	-1.3	1	1.8	-12.2

Table 4
Mean Maternal Food Consumption (Continued)

Food Consumption Mean

Sex: Female Day(s) Relative to Ma		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
18 → 20	Mean	25.3	24.0	26.4	27.1	25.1	24.1
	SD	3.6	1.5	3.3	2.1	3.4	2.7
	N	5	5	5	5	5	5
	%Diff	.	-5.2	4.3	7.1	-0.7	-4.8

Table 5
Summary of Maternal Gross Observations

	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
Number of Animals on Study :	5	5	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)	(5)
WHOLE BODY;						
Submitted	(0)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	5	5	5	5	4	5
LIVER;						
Submitted	(0)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	0	0	0
Discoloration; tan; left	0	0	0	0	1	0

Table 6
Reproductive Outcome

		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
Number of females with live foetuses at scheduled kill		5	5	5	5	5
Number of implantations		57	64	63	64	66
Number of implantations per female	Mean	11.4	12.8	12.6	12.8	13.1
Number of Intra-Uterine Deaths - Early Resorption		0	0	1	0	0
Number per female Including total resorptions	Mean	0.0	0.0	0.2	0.0	0.0
Number per female Excluding total resorptions	Mean	0.0	0.0	0.2	0.0	0.0
Post-implantation loss		0	0	1	0	0
Rate per female Including total resorptions	Mean	0.00	0.00	0.02	0.00	0.00
Rate per female Excluding total resorptions	Mean	0.00	0.00	0.02	0.00	0.00
% loss Including total resorptions		0.00	0.00	1.59	0.00	0.00
Excluding total resorptions		0.00	0.00	1.59	0.00	0.00
Number of live foetuses		57	64	62	64	66
Number per female Including total resorptions	Mean	11.3	12.8	12.4	12.8	13.1
Number per female Excluding total resorptions	Mean	11.4	12.8	12.4	12.8	13.1
% of implantations Including total resorptions		100.00	100.00	98.41	100.00	100.00
Excluding total resorptions		100.00	100.00	98.41	100.00	100.00

Statistical analysis not performed - Arithmetic mean values presented

Calculated values do not include animals which either, were not pregnant, did not survive to the scheduled kill, aborted or are marked for exclusion

Table 7
Mean Absolute and Relative Organ Weights in Female Rats

Sex: Female		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day	1000 mg/kg/day
Kidneys	Mean	1.97	1.99	2.03	2.16	2.11	2.05
Weight	SD	0.24	0.14	0.13	0.14	0.16	0.14
(g)	N	5	5	5	5	5	5
Kidney /Term	Mean	0.518	0.527	0.539	0.544	0.567	0.565
inal Bodywei	SD	0.042	0.025	0.038	0.027	0.042	0.031
	N	5	5	5	5	5	5
Liver	Mean	15.52	15.45	15.57	17.39	18.27	18.06
Weight	SD	1.70	1.11	1.12	1.23	1.05	1.26
(g)	N	5	5	5	5	5	5
Liver /Termi	Mean	4.086	4.101	4.122	4.375	4.923	4.990
nal Bodyweig	SD	0.290	0.238	0.108	0.259	0.333	0.252
	N	5	5	5	5	5	5

APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

-/---/./…/Blank space	-	no data/data could not be calculated
GD	-	gestation day
kg	-	kilogram
mg	-	milligram
N/n	-	number in group/Number of values used in calculation of mean
Wt	-	weight

NOTES:

Due to rounding differences, values in tables may be slightly different than appendices.

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28548
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	84%
Other Components	Water – 12.7% Perfluorooctanoic acid – 150 ppm
Date of Analysis	June 13, 2008
Expiration Date	June 13, 2011
Instructions for storage	NRT&H
Reference	DuPont-25455
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

24-JUN-2009
Date

Revision #1: Revised COA expiration date based on compound stability assessment. 6/23/09

Appendix B
Protocol

H-28548: Toxicokinetic Study in Pregnant Rats

Work Request Number 18405

Service Code 849

Protocol

Performing Laboratory: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers
for Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

Haskell Animal Welfare
Committee Number: DGRT201-P

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OBJECTIVE

The objective of the current study is to measure levels of the test substance, H-28548, in plasma prepared from blood collected from pregnant rats on gestation day (GD) 20 and from GD 20 fetuses.

SPONSOR AND CONTACT INFORMATION

Sponsor:	E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.
Sponsor Contact:	Jane Bradd Andersen 302-999-2377 Jane-Bradd.Andersen@usa.dupont.com
Testing Facility Contact:	Susan M. Munley 302-366-5240 Susan M. Munley@usa.dupont.com
Sponsor Approval:	found on the Work Authorization Form

REGULATORY COMPLIANCE

This study will be conducted in compliance with the following good laboratory practice(s), which are compatible with current OECD Good Laboratory Practices:

- U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards

ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for DuPont Haskell will adhere to the following principles:

- The sponsor and/or the study director ensures that the study described in this protocol does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or

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minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.

- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved will be painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee. The sponsor will be advised by the study director of all circumstances that could lead to this action in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- Animals will be provided with species-appropriate environmental enrichment.
- The procedures in this protocol have been reviewed by the Haskell Animal Welfare Committee and comply with acceptable standards for animal welfare and humane care.
- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

STUDY DESIGN

A. Treatment Groups and Daily Dosage

Group	Dosage (mg/kg/day)[a]	Formulation Concentration (mg/mL)[b]	Number of Timed Mated Females
1	0[c]	0	5
2	5	0.5	5
3	10	1	5
4	100	10	5
5	1000	100	5
6	1000	100	5

a Formulations of test substance in deionized water will be administered once daily by gavage on GD 6-20 at a dosing volume of 10 mL/kg.
b To achieve these concentrations of active ingredient, the formulations will be adjusted for sample purity.
c The control group animals will receive vehicle deionized water only at a dosing volume of 10 mL/kg.

B. Dosing and Sacrifice Schedules

Rats will be dosed once daily by oral gavage on GD 6 to 20 at the dose levels listed in the table above. The volume administered (10 mL/kg) will be based on the most recent body weight

Rats assigned to group 6 will be bled via tail vein on GD 6 two hours (± 5 minutes) following dosing.

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Rats assigned to groups 1 through 6 will be euthanized on GD 20 two hours (± 5 minutes) following dosing; blood will be collected at sacrifice and selected tissues will be weighed (livers, kidneys) and retained (livers, kidneys, ovaries, uterus).

A maximal volume of blood will be collected from each rat at sacrifice and split for preparation of plasma and serum samples.

C. Selection of Route of Administration and Dose Levels

The test substance formulations will be administered orally because previous developmental and reproductive toxicity studies[1,2,3] were conducted using the same route of exposure.

The doses selected for the current study are 0, 5, 10, 100, and 1000 mg/kg/day. These doses have been tested in the previously cited studies and the current study is intended to provide information regarding the relative internal doses relevant to the previous work.

MATERIALS AND METHODS

A. Vehicle

Name: deionized water

B. Test Substance

The test substance will be supplied by the sponsor. The test substance was assigned Haskell identification number 28548.

Available information on the purity, composition, contaminants, synonyms, CAS registry number, basic physical properties, hazards, and hazardous material classification(s) will be provided by the sponsor. The sponsor-reported purity is 84%.

C. Dosing Formulations

1. Preparation

Formulations of the test substance in the vehicle will be prepared and used within the period of established stability. Formulation stability has been established previously in a separate study[4] and demonstrated that the test substance formulations were stable at room temperature for up to 12 days at concentrations ranging from 0.01 to 100 mg/mL. Dosing formulations will be stored at room temperature until used. The method of mixing the test substance with the vehicle will be documented in the study records.

2. Sampling and Analysis

Samples of each formulation will be taken 2 times: near the beginning and end of the study. Analyses will address concentration verification.

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Samples will be analyzed by the DuPont Haskell Analytical Chemistry Group on the day the samples are collected. If samples cannot be analyzed at the specified times, they will be refrigerated until analyses can be conducted. On days samples are taken, the formulations remaining after dosing will be refrigerated for possible additional analysis. At the time of analysis, the samples will be diluted with water and analyzed by high-performance liquid chromatography (HPLC) with mass spectrometry detection (LC/MS). Detailed methods along with a description of the sample preparation procedure will be fully documented in the study records and described in the final report. Additional samples may be taken at the discretion of the study director or designee. Whenever additional samples are taken, a sample of the vehicle will be collected and refrigerated on the day of formulation preparation.

D. Test System

Species/Strain:	Crl:CD(SD) rat
Sex:	Female (nulliparous, timed mated, GD 0 = day mating confirmed)
Supplier:	Charles River Laboratories, Inc., Raleigh, North Carolina
Gestation Day at Arrival:	GD 2
Number Received:	30 received on June 3, 2010
Age at Arrival:	Approximately 86 days
Age at Start of Study:	Approximately 91 days
Weight at Arrival:	Approximately 201-225 grams The weight range is not a factor for the purposes of this study; therefore, deviations outside of this range are expected not to have any impact on the study. The body weight range will be documented in the study records and provided in the final report.
Identification:	Each animal will be identified by the supplier prior to shipping by marking the tail in indelible ink with a unique number from a list provided by DuPont Haskell. The tail marks will be verified routinely and darkened, if necessary, to ensure tracking of animal identity.

GD 0 body weights will be supplied by the vendor and included in the study records.

E. Justification for Animal Model

The rat was selected for this study because it is a preferred species for developmental toxicity testing as recommended by test guidelines. The Crl:CD(SD) strain was chosen because extensive background information is available from the literature, the supplier, and previous studies conducted at DuPont Haskell. This strain is also considered suitable relative to hardiness and incidence of spontaneous disease.

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F. Animal Husbandry

Housing: individually in solid bottom caging with bedding and nestlets as enrichment

Cage Rack Positioning: Cage racks will not be relocated within the animal room.

Climate: Temperature of 18-26°C (64-79°F)
Relative humidity of 30-70%
The relative humidity and temperature values in the housing rooms will be recorded daily in the study records.

Illumination: Artificial (fluorescent light) on an approximate 12-hour light/dark cycle.

Water: Tap water *ad libitum*

Feed: PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 (pellets) *ad libitum*.

Unless judged by the study director or the laboratory veterinarian to have significantly affected the results of the study, the relative humidity and temperature ranges in the housing rooms will not be included in the final report.

1. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

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G. Quarantine and Pretest Periods

Rats will be quarantined for at least 3 days according to procedures outlined in DuPont Haskell Standard Operating Procedure (SOP) LA003-P, and then released for the study upon approval of the animal resources supervisor or designee.

H. Assignment of Animals to Groups

Before dosing begins, animals will be randomly assigned to control or experimental groups using a computerized randomization procedure to produce a homogeneous distribution of body weights across groups within each breeding lot. Animals that lose excessive weight or that are ill prior to the start of dosing will be removed from the study, sacrificed and discarded without pathological evaluation.

I. In-life Observations

Procedure	Frequency
Quarantine and Pretest	
Mortality/Moribundity	At least once daily
Clinical Observations	GD 4
Body Weights	GD 4
Food consumption	GD 4
Testing Period	
Mortality/Moribundity	Twice daily (AM and PM)
Blood Collection	For animals in group 6, once 2 hours (± 5 minutes) post-dosing on GD 6
Clinical Observations	Twice daily on GD 6-19 (during weighing and at least 2 hours post-dosing) Once on GD 20 Clinical signs observed at other times will be recorded by exception.
Body Weights	Daily on GD 6-20
Food Consumption	GD 6, 8, 10, 12, 14, 16, 18, and 20

J. Animal Euthanasia

Females will be euthanized by carbon dioxide asphyxiation and exsanguination; a maximal volume of blood will be collected. The whole blood will be divided so that both plasma and serum can be prepared.

Fetuses will be euthanized by decapitation and trunk blood will be collected and pooled by litter. Fetal plasma will be prepared.

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K. Postmortem Evaluations

1. Females Dying Prior to Scheduled Euthanasia (Found Dead)

A gross external and visceral examination will be performed in a timely manner.

Animals that are found dead will be refrigerated until a necropsy can be performed. For each found dead animal, the liver and kidneys will be retained for possible future examination. In addition, gross lesions will be retained for possible future examination; lesions for which a microscopic diagnosis would not be additive (e.g., osteoarthritis, pododermatitis, tail chronic dermatitis, calculus, and deformities of the teeth, toe, tail, or ear pinnae) will not be saved. Pregnancy status will be recorded. Grossly abnormal uterine contents will be recorded as maternal gross postmortem findings and will be retained for further examination at the discretion of the study director or designee. Uteri with no visible implantation sites will be placed in a 10% aqueous solution of ammonium sulfide[5] to detect very early resorptions.

2. Females Delivering Early and Females Euthanized Prior to Scheduled Termination

Dams that deliver before scheduled euthanasia and dams that are euthanized prior to scheduled termination will be euthanized and a gross external and visceral examination performed in a timely manner. For each maternal animal and for fetuses delivered early and remaining *in utero*, blood will be collected and prepared to plasma and serum. In addition, the weight of the liver and kidneys will be recorded. For each female, kidneys, a portion of the liver, and one ovary will be placed in formalin. The remainder of the liver, uterus, and the remaining ovary will be flash frozen in liquid nitrogen and stored between -60ºC and -80ºC. These tissues will be retained for possible future examination. Any additional examination of these tissues as well as the disposition of these tissues will be provided in a protocol amendment.

Gross lesions will be retained for possible histologic examination; lesions for which a microscopic diagnosis would not be additive (e.g., osteoarthritis, pododermatitis, tail chronic dermatitis, calculus, and deformities of the teeth, toe, tail, or ear pinnae) will not be saved.

Grossly abnormal uterine contents will be recorded by exception as maternal gross postmortem findings and will be retained for further examination at the discretion of the study director or designee.

The uterine horns from uteri with no visible implantation sites will be placed in a 10% aqueous solution of ammonium sulfide[5] to detect very early resorptions. The uterine body will be frozen for possible future evaluation.

3. Females Surviving to Scheduled Euthanasia

A gross external and visceral examination will be performed immediately after euthanasia. Gross lesions will be retained for possible histologic examination; lesions for which a microscopic diagnosis would not be additive (e.g., osteoarthritis, pododermatitis, tail chronic dermatitis, calculus, and deformities of the teeth, toe, tail, or ear pinnae) will not be saved.

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The uterine horns from uteri with no visible implantation sites will be placed in a 10% aqueous solution of ammonium sulfide[5] to detect very early resorptions. The uterine body will be frozen for possible future evaluation.

For each animal, blood will be collected and prepared to plasma and serum. In addition, the weight of the liver and kidneys will be recorded. For each female, kidneys, a portion of the liver, and one ovary will be placed in formalin. The remainder of the liver, uterus, and the remaining ovary will be flash frozen in liquid nitrogen and stored between -60°C and -80°C. These tissues will be retained for possible future histopathologic examination. Any additional examination of these tissues as well as the disposition of these tissues will be provided in a protocol amendment.

For each female with visible implantation sites, the types of implantations (live and dead fetuses, early and late resorptions) and their relative positions in the uterus will be recorded. Live fetuses will be decapitated to collect trunk blood which will be pooled by litter and prepared to plasma.

The types of implantations are classified as follows:

live fetus:	fully formed and responds to stimuli
dead fetus:	fully formed with little or no evidence of maceration
late resorption:	identifiable structures (i.e. digital rays)
early resorption:	no visible fetal structures

L. Blood Collection and Plasma Analysis

Whole blood will be collected from rats via tail vein in Group 6 two hours (± 5 minutes) after dosing on GD 6. The actual time of blood collection will be recorded. Whole blood from the animals in group 6 as well as from all other groups 1 through 5 will be collected from the vena cava at sacrifice. Whole blood from fetuses will be collected from the trunk following decapitation and pooled by litter.

Whole blood samples that will be used for analytical analysis will be collected using EDTA or heparin as an anticoagulant and processed to plasma by centrifugation. The red blood cell fraction will be discarded after separation. Plasma will be stored frozen at ≤ 20°C until delivery to the DuPont Haskell Analytical Chemistry Group.

Samples will be analyzed for the test substance using LC-MS-MS. The analytical method used will be documented in the study records and will be included in the final report. The contact for analytical analysis is:

Michael Mawn, Ph.D.
Michael.P.Mawn@usa.dupont.com
302-451-3365

M. Serum Preparation and Storage

A minimum of 0.6 mL of whole blood will be placed in a serum separator tube on ice until the serum is prepared. Serum will be stored between -60°C and -80°C for possible future analysis.

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Analysis as well as the disposition of the serum will be at the discretion of the study director and will be provided for in a protocol amendment. Any analyses and sample disposition will be documented in the study records.

N. Ovary/Uterus Collection and Storage

For each rat, one ovary and the uterus will be collected and flash frozen in liquid nitrogen and stored between -60°C and -80°C for possible future analysis. Analyses as well as the disposition of these tissues will be provided for in a protocol amendment, will be performed at the discretion of the study director and will be documented in the study records.

STATISTICAL ANALYSES

Descriptive statistics will be performed on endpoints evaluated for this study. Other methods will be used if appropriate, at the time of analysis. The statistical methods used will be described in the final report.

SAFETY AND HOUSEKEEPING

Good housekeeping procedures will be practiced to avoid contamination of dosing formulation preparation facilities and potential health hazards. To avoid skin contact, gloves will be worn when handling the test substance or dosing formulations. In addition, the test substance will be handled in a chemical hood. Dosing formulations will be prepared in properly ventilated areas. Animal carcasses and feces will be incinerated.

RECORDS AND SAMPLE STORAGE

All raw data, the protocol, amendments (if any), and the final report will be retained.

STUDY DATES

Proposed Experimental Start: June 7, 2010

Proposed In-life Completion Date: June 21, 2010

Proposed Experimental Termination: September 8, 2010

REFERENCES

1. WIL Research Laboratories, LLC (in progress). An Oral (Gavage) Prenatal Developmental Toxicity Study of H-28548 in Rats. Unpublished report, DuPont-18405-841.

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H-28548:
Toxicokinetic Study in Pregnant Rats
DuPont-18405-849

SIGNATURES

Approved by: Susan M. Munley 4 June 2010
Susan M. Munley, M.A. Date
Study Director

Appendix C
Analysis for H-28548 in Dose Samples

ANALYSIS FOR H-28548 IN DOSE SAMPLES

for

H-28548: Toxicokinetic Study in Pregnant Rats

Work Request Number:	18405
Service Code:	849
Haskell Sample Number:	28548
Analytical Report Number:	DuPont-18405-849-AN
DuPont Study Number:	DuPont-18405-849
Notebook:	E-98517-PB

SUMMARY

Dose samples of H-28548 at the concentrations of 0.5, 1, 10, and 100 mg/mL prepared on June 04, 2010 and June 14, 2010 were submitted for concentration verification analysis. A 0 mg/mL control sample containing the vehicle only, deionized (DI) water, was included and analyzed together with each set of samples.

Samples were diluted with water and concentrations of H-28548 in the diluted samples were determined by high-performance liquid chromatography (HPLC) with mass spectrometry detection (LC/MS).

The analysis results show that the test substance was at the targeted concentrations for the dose levels in the vehicle for the study.

Test substance was not detected in the 0 mg/mL control samples.

MATERIALS AND METHODS

All solvents used were HPLC grade.

1. Dose Sample Preparation

An aliquot of 0.5 mL of the dose samples was initially diluted with water to a total of 50 mL. The diluted 0 and 0.5 mg/mL samples were analyzed by LC/MD directly while other dose samples were further diluted with water to a nominal concentration of 0.005 mg/mL before LC/MS analysis.

2. Chromatographic Conditions

Concentrations of H-28548 in diluted dose samples were determined by LC/MS.

LC Parameters

Instrument:	Agilent 1100 liquid chromatograph
Column:	Agilent Zorbax® SB-C8, 2.1 x 100 mm, 3.5 µm
Mobile Phase:	50% of 20 mM Ammonium acetate in water 50% of 20 mM Ammonium acetate in methanol
Flow Rate:	0.400 mL/min
Stop Time:	5.0 min
Column Temperature:	35°C
Injection Volume:	2.0 µL

MS Parameters

Instrument:	Waters Micromass ZQ
Function Type:	SIR mass: 329 m/z
Ion Mode:	Electrospray, negative (ES-)
Capillary Voltage:	3.20 kV
Cone Voltage:	9.00 V
Source Temperature:	120°C
Desolvation Temperature:	300°C
Cone Gas Flow:	40 L/Hr
Desolvation Gas Flow:	400 L/Hr

3. Calibration and Quantitation

The test substance (H-28548, 84%) was used as the analytical reference standard to make a stock solution in water. The stock solution was further diluted with water to make a set of standard solutions. These standard solutions were then used to generate a calibration curve that covered the targeted concentration of the diluted samples.

Peak areas from the LC/MS analysis of these standard solutions were used to construct a calibration curve with a quadratic fit by Waters Masslynx software (see Figure 1 for a

representative curve). Measured concentrations for the diluted dose samples were determined by applying the peak areas from replicate injections of each sample to the calibration curve.

Concentration verification of the test substance in the dose samples was evaluated by the average results of the duplicate sample analyses for each respective dose level. Relative standard deviation (RSD = standard deviation/average x 100) was calculated to confirm the concentrations.

RESULTS AND DISCUSSION

1. Chromatography

H-28548 eluted from the HPLC column as a resolved peak with a retention time of approximately 3.25 minutes. Representative HPLC chromatograms are shown in Figures 2a - c. Test substance was not detected in the control dose samples.

2. Sample Analysis Results

Detailed results for the concentration verification analysis of the dose samples are shown in Table 1.

The data for the dose samples prepared on June 04, 2010 and June 14, 2010 show that the test substance was at the targeted concentrations (± 5.1% of nominal and RSDs ≤ 4%) in the vehicle for the study. Test substance was not detected in the control samples.

3. Conclusions

The analytical results show that the test substance was at the targeted concentrations of the dose levels in the vehicle for the study. Test substance was not detected in the 0 mg/mL control samples.

Table 1: Analysis Results of H-28548 in Dose Samples

Formulation Date	H-28548 (mg/mL)		Percent
	Nominal	Measured	Nominal
04-June-2010			
Control	0	ND[A]	---
1	0.5	0.487	97.4
2	0.5	0.499	99.8
	Average ± SD[B]:	***0.493 ± 0.008***	***(98.6)***
	RSD[B]:	***2%***	
1	1	1.06	106.0
2	1	1.03	103.0
	Average ± SD:	***1.05 ± 0.02***	***(105.0)***
	RSD:	***2%***	
1	10	9.65	96.5
2	10	10.2	102.0
	Average ± SD:	***9.93 ± 0.39***	***(99.3)***
	RSD:	***4%***	
1	100	105	105.0
2	100	102	102.0
	Average ± SD:	***104 ± 2***	***(104.0)***
	RSD:	***2%***	
14-June-2010			
Control	0	ND	---
1	0.5	0.476	95.2
2	0.5	0.487	97.4
	Average ± SD:	***0.482 ± 0.008***	***(96.4)***
	RSD:	***2%***	
1	1	0.939	93.9
2	1	0.999	99.9
	Average ± SD:	***0.969 ± 0.042***	***(96.9)***
	RSD:	***4%***	
1	10	9.79	97.9
2	10	9.39	93.9
	Average ± SD:	***9.59 ± 0.28***	***(95.9)***
	RSD:	***3%***	
1	100	93.9	93.9
2	100	95.9	95.9
	Average ± SD:	***94.9 ± 1.4***	***(94.9)***
	RSD:	***1%***	

[A] ND = Not Detected.

[B] Concentration for each dose level is the average of the duplicate analysis results; SD (standard deviation) and RSD calculated to confirm concentrations. The percent nominal of the average is shown in parenthesis.

Figure 1
Representative Calibration Curve

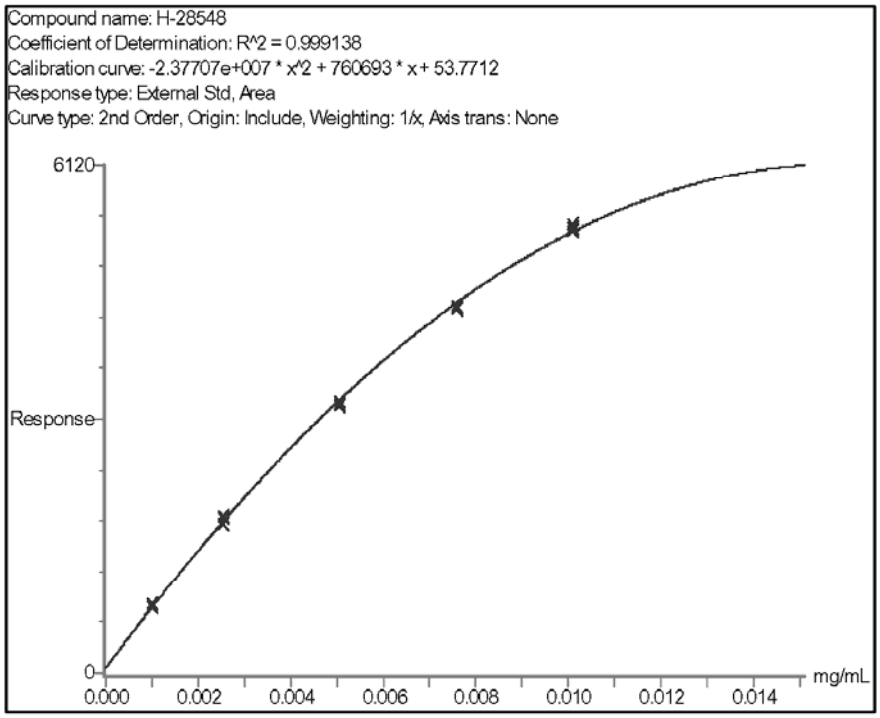

Figure 1. Representative calibration curve showing linear fit to replicate peak area measurements for calibration standard solutions of H-28548 over the concentration range from 0.00101 to 0.0101 mg/mL.

Figure 2
Representative LC/MS Chromatograms

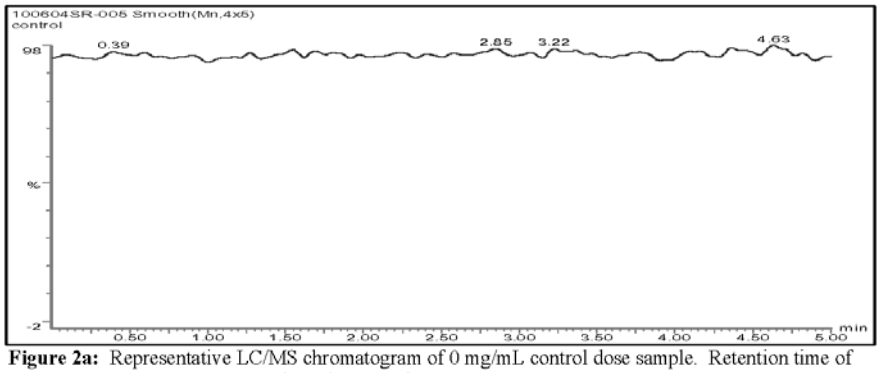

Figure 2a: Representative LC/MS chromatogram of 0 mg/mL control dose sample. Retention time of H-28548 was approximately 3.25 minutes.

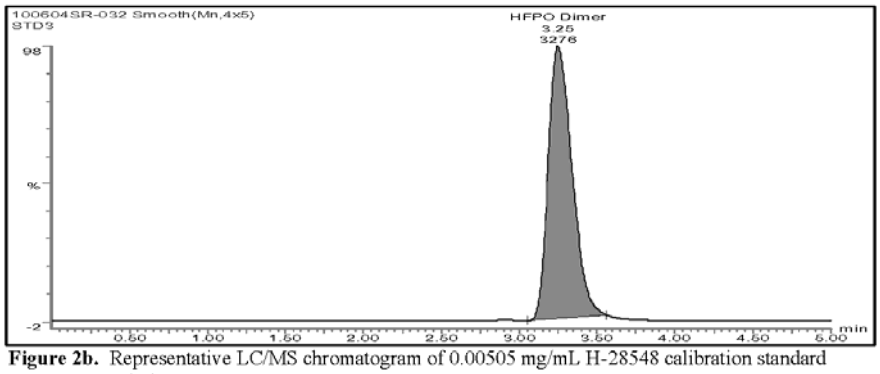

Figure 2b. Representative LC/MS chromatogram of 0.00505 mg/mL H-28548 calibration standard solution.

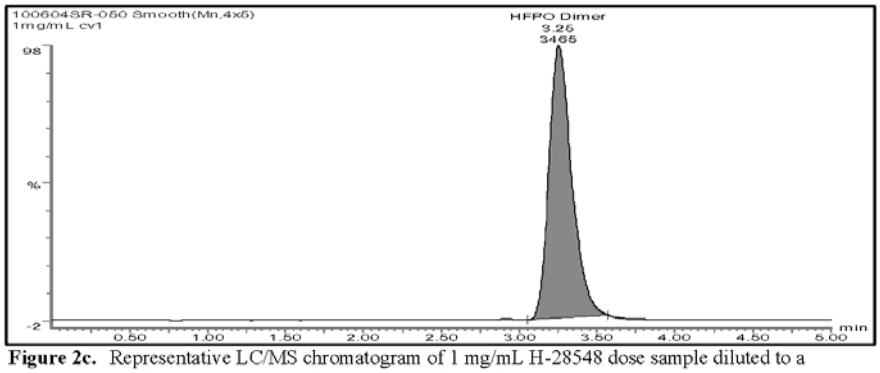

Figure 2c. Representative LC/MS chromatogram of 1 mg/mL H-28548 dose sample diluted to a nominal concentration of 0.005 mg/mL for analysis.

Appendix D
Individual Maternal Clinical Observations

INDIVIDUAL MATERNAL CLINICAL OBSERVATIONS

EXPLANATORY NOTES

ABBREVIATIONS:

X - present

NOTES:

Clinical observations and fates are recorded in the following time slots:

A - careful (with body weights or pre-treatment) and mode of death
B - careful post-treatment

Individual Maternal Clinical Observations

Day numbers relative to Mating Date

Group	Sex	Animal	Clinical Sign	Site	6 A	6 B	7 A	7 B	8 A	8 B	9 A	9 B	10 A	10 B	11 A	11 B	12 A	12 B	13 A	13 B	14 A	14 B
1	f	101	No Abnormalities Detected		X	X	X	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			Hair loss	Forelimb bilateral	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Hair loss	Inguen	.	.	.	.	.	.	X	X	X	X	X	X	X	X	X	X	X	X
		102	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		103	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		104	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		105	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	f	201	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		202	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		203	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		204	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		205	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	f	301	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			Mass	Abdomen	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			Comment Present		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		302	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		303	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		304	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		305	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Group 1 - 0 mg/kg/day Group 2 - 5 mg/kg/day Group 3 - 10 mg/kg/day
Group 4 - 100 mg/kg/day Group 5 - 1000 mg/kg/day Group 6 - 1000 mg/kg/day

Individual Maternal Clinical Observations

Day numbers relative to Mating Date

Group	Sex	Animal	Clinical Sign	Site	15 A	15 B	16 A	16 B	17 A	17 B	18 A	18 B	19 A	19 B	20 A
1	f	101	No Abnormalities Detected		.	.	.	.	.	.	.	.	.	.	.
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
			Hair loss	Forelimb bilateral	X	X	X	X	X	X	X	X	X	X	X
			Hair loss	Inguen	X	X	X	X	X	X	X	X	X	X	X
		102	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		103	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		104	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		105	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
2	f	201	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		202	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		203	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		204	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		205	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
3	f	301	No Abnormalities Detected		X	.	.	.	.	.	.	.	.	.	.
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
			Mass	Abdomen	.	X	X	X	X	X	X	X	X	X	X
			Comment Present		.	*	*	*	*	*	*	*	*	*	*
		302	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		303	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		304	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		305	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X

Group 1 - 0 mg/kg/day Group 2 - 5 mg/kg/day Group 3 - 10 mg/kg/day
Group 4 - 100 mg/kg/day Group 5 - 1000 mg/kg/day Group 6 - 1000 mg/kg/day

Individual Maternal Clinical Observations

Day numbers relative to Mating Date

Group	Sex	Animal	Clinical Sign	Site	6 A	6 B	7 A	7 B	8 A	8 B	9 A	9 B	10 A	10 B	11 A	11 B	12 A	12 B	13 A	13 B	14 A	14 B
4	f	401	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		402	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		403	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			Wet fur	Perineum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		404	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		405	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	f	501	No Abnormalities Detected		X	X	X	X	X	.	.	.	.	X	X	X	X	X	X	.	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			Wet fur	Perineum	.	.	.	.	.	X	X	X	X	.	.	.	.	.	.	X	.	.
		502	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		503	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			Wet fur	Perineum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		504	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		505	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	f	601	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		602	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		603	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		604	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		605	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Group 1 - 0 mg/kg/day Group 2 - 5 mg/kg/day Group 3 - 10 mg/kg/day
Group 4 - 100 mg/kg/day Group 5 - 1000 mg/kg/day Group 6 - 1000 mg/kg/day

Individual Maternal Clinical Observations

Day numbers relative to Mating Date

Group	Sex	Animal	Clinical Sign	Site	15 A	15 B	16 A	16 B	17 A	17 B	18 A	18 B	19 A	19 B	20 A
4	f	401	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		402	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		403	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
			Wet fur	Perineum	.	.	.	.	.	.	.	.	.	.	.
		404	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		405	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
5	f	501	No Abnormalities Detected		X	X	X	.	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
			Wet fur	Perineum	.	.	.	X	.	.	.	.	.	.	.
		502	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		503	No Abnormalities Detected		X	X	X	.	.	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
			Wet fur	Perineum	.	.	.	X	X	.	.	.	.	.	.
		504	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		505	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
6	f	601	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		602	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		603	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		604	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X
		605	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	X

Group 1 - 0 mg/kg/day Group 2 - 5 mg/kg/day Group 3 - 10 mg/kg/day
Group 4 - 100 mg/kg/day Group 5 - 1000 mg/kg/day Group 6 - 1000 mg/kg/day

Individual Maternal Clinical Observations

Group	Sex	Animal	Day Number	Time Slot	Comments: Comment
3	f	301	15	B	firm, 5 mm dia.
			16	A	firm, 5 mm dia.
				B	firm, 5 mm dia.
			17	A	firm, 5 mm dia.
				B	firm, 5 mm dia.
			18	A	firm, 5 mm dia.
				B	firm, 5 mm dia.
			19	A	firm, 5 mm dia.
				B	firm, 5 mm dia.
			20	A	firm, 5 mm dia.

Appendix E
Individual Maternal Body Weights

INDIVIDUAL MATERNAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

N - number of values used in calculation
SD - standard deviation

Individual Maternal Body Weights (g)

Sex: Female — Bodyweight (g)

0 mg/kg/day	Day(s) Relative to Mating (L)						
	6	7	8	9	10	11	12
101	276.9	281.8	280.4	285.6	264.3	252.9	293.9
102	260.8	266.4	270.7	245.2	281.3	286.8	288.6
103	297.1	303.1	307.4	311.6	326.3	332.0	337.2
104	264.6	270.4	269.2	273.2	252.7	241.8	287.8
105	259.6	254.6	256.2	260.4	270.7	279.2	290.6
Mean	271.8	275.3	276.8	275.2	279.1	278.5	299.6
SD	15.7	18.3	19.2	25.3	28.4	35.1	21.1
N	5	5	5	5	5	5	5

Sex: Female — Bodyweight (g)

5 mg/kg/day	Day(s) Relative to Mating (L)						
	6	7	8	9	10	11	12
201	264.8	269.3	268.2	273.2	279.0	290.9	291.7
202	254.5	258.9	266.8	269.7	277.4	288.1	295.7
203	281.6	280.8	287.5	292.6	292.5	308.6	313.3
204	273.7	277.8	280.7	291.1	297.0	308.2	310.4
205	263.2	268.7	272.6	274.1	277.6	290.9	304.2
Mean	267.6	271.1	275.2	280.1	284.7	297.3	303.1
SD	10.4	8.6	8.8	10.8	9.3	10.2	9.3
N	5	5	5	5	5	5	5

Individual Maternal Body Weights (g)

Sex: Female

Bodyweight (g)

10 mg/kg/day	Day(s) Relative to Mating (L)						
	6	7	8	9	10	11	12
301	266.4	274.8	279.1	284.9	293.3	303.1	310.2
302	264.7	264.1	262.0	274.0	284.2	294.4	302.4
303	290.3	295.2	294.5	296.7	307.1	316.0	327.0
304	244.0	242.4	237.6	238.0	257.4	270.2	267.3
305	258.1	253.5	259.6	260.5	262.9	270.3	282.0
Mean	264.7	266.0	266.6	270.8	281.0	290.8	297.8
SD	16.8	20.3	21.5	22.7	20.8	20.3	23.5
N	5	5	5	5	5	5	5

Sex: Female

Bodyweight (g)

100 mg/kg/day	Day(s) Relative to Mating (L)						
	6	7	8	9	10	11	12
401	271.6	272.7	277.3	283.0	287.0	296.2	298.7
402	268.7	277.3	278.5	287.5	296.7	308.2	309.5
403	283.7	292.7	291.8	301.0	310.7	322.3	329.1
404	267.1	275.3	278.9	285.0	298.5	302.1	309.8
405	287.9	289.7	288.2	285.4	301.2	305.0	303.9
Mean	275.8	281.5	282.9	288.4	298.8	306.8	310.2
SD	9.4	9.0	6.6	7.2	8.5	9.7	11.5
N	5	5	5	5	5	5	5

Individual Maternal Body Weights (g)

Sex: Female — Bodyweight (g)

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	6	7	8	9	10	11	12
501	274.1	273.0	281.4	283.0	286.6	283.5	289.8
502	245.0	250.4	249.7	253.3	257.5	266.3	273.7
503	288.5	288.2	287.7	287.1	289.6	305.1	308.4
504	253.2	251.1	252.2	248.4	252.1	267.3	271.2
505	292.2	282.7	281.8	286.2	300.6	313.2	315.7
Mean	270.6	269.1	270.6	271.6	277.3	287.1	291.8
SD	21.0	17.6	18.1	19.1	21.3	21.5	20.0
N	5	5	5	5	5	5	5

Sex: Female — Bodyweight (g)

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	6	7	8	9	10	11	12
601	261.9	258.9	263.4	265.4	272.2	279.0	284.3
602	255.4	242.6	250.7	249.8	257.3	263.7	267.9
603	290.0	282.5	290.8	291.1	300.5	307.5	312.4
604	251.6	239.3	246.5	247.7	256.1	262.4	271.3
605	280.9	276.7	277.8	283.1	288.3	294.1	293.5
Mean	268.0	260.0	265.8	267.4	274.9	281.3	285.9
SD	16.7	19.5	18.5	19.4	19.4	19.5	18.0
N	5	5	5	5	5	5	5

Individual Maternal Body Weights (g)

Sex: Female — Bodyweight (g)

0 mg/kg/day	Day(s) Relative to Mating (L)						
	13	14	15	16	17	18	19
101	292.7	300.3	303.8	311.1	326.9	344.4	358.3
102	294.3	298.0	304.4	306.0	320.6	330.9	342.5
103	344.8	352.3	357.0	367.1	381.6	396.6	406.9
104	292.2	297.6	309.3	318.6	341.8	355.7	371.7
105	289.3	295.6	302.0	302.8	321.2	335.3	363.6
Mean	302.7	308.8	315.3	321.1	338.4	352.6	368.6
SD	23.6	24.4	23.5	26.4	25.6	26.4	23.9
N	5	5	5	5	5	5	5

Sex: Female — Bodyweight (g)

5 mg/kg/day	Day(s) Relative to Mating (L)						
	13	14	15	16	17	18	19
201	295.3	297.2	305.1	308.1	322.9	333.4	349.3
202	297.7	304.1	310.1	319.0	333.6	349.2	364.3
203	313.8	314.3	321.8	322.8	350.2	361.4	375.8
204	302.9	315.2	317.4	327.8	347.9	363.7	378.4
205	310.0	310.2	310.8	313.1	312.3	310.8	336.8
Mean	303.9	308.2	313.0	318.2	333.4	343.7	360.9
SD	7.9	7.6	6.6	7.8	16.2	22.0	17.7
N	5	5	5	5	5	5	5

Individual Maternal Body Weights (g)

Sex: Female — Bodyweight (g)

10 mg/kg/day	Day(s) Relative to Mating (L)						
	13	14	15	16	17	18	19
301	316.0	317.1	324.3	331.6	351.5	358.6	374.5
302	304.1	303.0	310.0	320.8	334.2	352.7	367.9
303	333.0	342.8	348.4	359.2	378.9	392.9	409.0
304	273.9	278.0	279.8	289.3	299.0	319.4	333.7
305	283.2	280.5	295.3	301.0	308.0	329.6	344.1
Mean	302.0	304.3	311.6	320.4	334.3	350.6	365.8
SD	24.0	27.0	26.4	27.3	32.5	28.6	29.4
N	5	5	5	5	5	5	5

Sex: Female — Bodyweight (g)

100 mg/kg/day	Day(s) Relative to Mating (L)						
	13	14	15	16	17	18	19
401	303.8	310.7	315.7	323.4	337.9	359.4	370.7
402	316.9	319.2	331.0	335.1	355.1	369.6	389.2
403	335.0	335.7	345.1	361.9	366.4	383.1	400.7
404	316.9	317.0	326.7	336.1	352.7	370.2	380.1
405	315.9	321.0	327.4	338.9	353.1	372.7	384.7
Mean	317.7	320.7	329.2	339.1	353.0	371.0	385.1
SD	11.1	9.2	10.6	14.1	10.1	8.5	11.1
N	5	5	5	5	5	5	5

Individual Maternal Body Weights (g)

Sex: Female

Bodyweight (g)

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	13	14	15	16	17	18	19
501	291.0	301.3	308.8	318.2	337.8	358.1	376.7
502	280.7	281.0	291.5	298.8	315.5	341.2	347.7
503	317.9	326.9	333.4	341.4	362.9	376.5	381.3
504	277.7	279.3	289.5	302.1	318.5	339.6	349.9
505	315.1	324.0	326.1	331.6	349.5	363.4	381.4
Mean	296.5	302.5	309.9	318.4	336.8	355.8	367.4
SD	19.0	22.7	19.8	18.4	20.2	15.5	17.1
N	5	5	5	5	5	5	5

Sex: Female

Bodyweight (g)

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	13	14	15	16	17	18	19
601	288.5	294.6	303.2	316.5	328.7	347.3	366.7
602	268.7	275.8	273.3	285.1	292.2	310.1	324.3
603	317.1	322.9	327.6	333.2	344.9	362.6	375.4
604	277.0	284.5	291.5	302.5	317.8	342.8	354.6
605	297.6	297.4	309.3	317.3	334.3	349.6	368.1
Mean	289.8	295.0	301.0	310.9	323.6	342.5	357.8
SD	18.8	17.8	20.2	18.1	20.1	19.5	20.2
N	5	5	5	5	5	5	5

Individual Maternal Body Weights (g)

Sex: Female

Bodyweight (g)

0 mg/kg/day	Day(s) Relative to Mating (L)
	20
101	366.9
102	351.4
103	424.2
104	386.9
105	368.4
Mean	379.6
SD	27.9
N	5

Sex: Female

Bodyweight (g)

5 mg/kg/day	Day(s) Relative to Mating (L)
	20
201	357.5
202	383.6
203	386.7
204	396.1
205	359.9
Mean	376.8
SD	17.1
N	5

Individual Maternal Body Weights (g)

Sex: Female

Bodyweight (g)

10 mg/kg/day	Day(s) Relative to Mating (L)
	20
301	387.1
302	382.0
303	419.5
304	347.6
305	353.0
Mean	377.8
SD	29.0
N	5

Sex: Female

Bodyweight (g)

100 mg/kg/day	Day(s) Relative to Mating (L)
	20
401	379.4
402	398.2
403	411.3
404	398.4
405	399.9
Mean	397.4
SD	11.5
N	5

Individual Maternal Body Weights (g)

Sex: Female

Bodyweight (g)

1000 mg/kg/day	Day(s) Relative to Mating (L)
	20
501	382.1
502	356.5
503	377.4
504	360.3
505	381.6
Mean	371.6
SD	12.2
N	5

Sex: Female

Bodyweight (g)

1000 mg/kg/day	Day(s) Relative to Mating (L)
	20
601	368.3
602	327.1
603	371.4
604	370.2
605	373.7
Mean	362.1
SD	19.7
N	5

Appendix F
Individual Maternal Body Weight Gains

INDIVIDUAL MATERNAL BODY WEIGHT GAINS

EXPLANATORY NOTES

ABBREVIATIONS:

N - number of values used in calculation
SD - standard deviation

Individual Maternal Body Weight Gains (g)

Sex: Female — Body Weight Gain

0 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 7	7 → 8	8 → 9	9 → 10	10 → 11	11 → 12	12 → 13
101	4.9	-1.4	5.2	-21.3	-11.4	41.0	-1.2
102	5.6	4.3	-25.5	36.1	5.5	1.8	5.7
103	6.0	4.3	4.2	14.7	5.7	5.2	7.6
104	5.8	-1.2	4.0	-20.5	-10.9	46.0	4.4
105	-5.0	1.6	4.2	10.3	8.5	11.4	-1.3
Mean	3.5	1.5	-1.6	3.9	-0.5	21.1	3.0
SD	4.7	2.8	13.4	24.6	9.8	20.8	4.1
N	5	5	5	5	5	5	5

Sex: Female — Body Weight Gain

5 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 7	7 → 8	8 → 9	9 → 10	10 → 11	11 → 12	12 → 13
201	4.5	-1.1	5.0	5.8	11.9	0.8	3.6
202	4.4	7.9	2.9	7.7	10.7	7.6	2.0
203	-0.8	6.7	5.1	-0.1	16.1	4.7	0.5
204	4.1	2.9	10.4	5.9	11.2	2.2	-7.5
205	5.5	3.9	1.5	3.5	13.3	13.3	5.8
Mean	3.5	4.1	5.0	4.6	12.6	5.7	0.9
SD	2.5	3.5	3.4	3.0	2.2	5.0	5.1
N	5	5	5	5	5	5	5

Individual Maternal Body Weight Gains (g)

Sex: Female — Body Weight Gain

10 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 7	7 → 8	8 → 9	9 → 10	10 → 11	11 → 12	12 → 13
301	8.4	4.3	5.8	8.4	9.8	7.1	5.8
302	-0.6	-2.1	12.0	10.2	10.2	8.0	1.7
303	4.9	-0.7	2.2	10.4	8.9	11.0	6.0
304	-1.6	-4.8	0.4	19.4	12.8	-2.9	6.6
305	-4.6	6.1	0.9	2.4	7.4	11.7	1.2
Mean	1.3	0.6	4.3	10.2	9.8	7.0	4.3
SD	5.2	4.5	4.8	6.1	2.0	5.9	2.6
N	5	5	5	5	5	5	5

Sex: Female — Body Weight Gain

100 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 7	7 → 8	8 → 9	9 → 10	10 → 11	11 → 12	12 → 13
401	1.1	4.6	5.7	4.0	9.2	2.5	5.1
402	8.6	1.2	9.0	9.2	11.5	1.3	7.4
403	9.0	-0.9	9.2	9.7	11.6	6.8	5.9
404	8.2	3.6	6.1	13.5	3.6	7.7	7.1
405	1.8	-1.5	-2.8	15.8	3.8	-1.1	12.0
Mean	5.7	1.4	5.4	10.4	7.9	3.4	7.5
SD	3.9	2.7	4.9	4.5	4.0	3.7	2.7
N	5	5	5	5	5	5	5

Individual Maternal Body Weight Gains (g)

Sex: Female — Body Weight Gain

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 7	7 → 8	8 → 9	9 → 10	10 → 11	11 → 12	12 → 13
501	-1.1	8.4	1.6	3.6	-3.1	6.3	1.2
502	5.4	-0.7	3.6	4.2	8.8	7.4	7.0
503	-0.3	-0.5	-0.6	2.5	15.5	3.3	9.5
504	-2.1	1.1	-3.8	3.7	15.2	3.9	6.5
505	-9.5	-0.9	4.4	14.4	12.6	2.5	-0.6
Mean	-1.5	1.5	1.0	5.7	9.8	4.7	4.7
SD	5.3	3.9	3.3	4.9	7.7	2.1	4.2
N	5	5	5	5	5	5	5

Sex: Female — Body Weight Gain

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 7	7 → 8	8 → 9	9 → 10	10 → 11	11 → 12	12 → 13
601	-3.0	4.5	2.0	6.8	6.8	5.3	4.2
602	-12.8	8.1	-0.9	7.5	6.4	4.2	0.8
603	-7.5	8.3	0.3	9.4	7.0	4.9	4.7
604	-12.3	7.2	1.2	8.4	6.3	8.9	5.7
605	-4.2	1.1	5.3	5.2	5.8	-0.6	4.1
Mean	-8.0	5.8	1.6	7.5	6.5	4.5	3.9
SD	4.5	3.1	2.3	1.6	0.5	3.4	1.8
N	5	5	5	5	5	5	5

Individual Maternal Body Weight Gains (g)

Sex: Female — Body Weight Gain

0 mg/kg/day	Day(s) Relative to Mating (L)						
	13 → 14	14 → 15	15 → 16	16 → 17	17 → 18	18 → 19	19 → 20
101	7.6	3.5	7.3	15.8	17.5	13.9	8.6
102	3.7	6.4	1.6	14.6	10.3	11.6	8.9
103	7.5	4.7	10.1	14.5	15.0	10.3	17.3
104	5.4	11.7	9.3	23.2	13.9	16.0	15.2
105	6.3	6.4	0.8	18.4	14.1	28.3	4.8
Mean	6.1	6.5	5.8	17.3	14.2	16.0	11.0
SD	1.6	3.1	4.3	3.7	2.6	7.2	5.1
N	5	5	5	5	5	5	5

Sex: Female — Body Weight Gain

5 mg/kg/day	Day(s) Relative to Mating (L)						
	13 → 14	14 → 15	15 → 16	16 → 17	17 → 18	18 → 19	19 → 20
201	1.9	7.9	3.0	14.8	10.5	15.9	8.2
202	6.4	6.0	8.9	14.6	15.6	15.1	19.3
203	0.5	7.5	1.0	27.4	11.2	14.4	10.9
204	12.3	2.2	10.4	20.1	15.8	14.7	17.7
205	0.2	0.6	2.3	-0.8	-1.5	26.0	23.1
Mean	4.3	4.8	5.1	15.2	10.3	17.2	15.8
SD	5.1	3.3	4.2	10.4	7.0	4.9	6.1
N	5	5	5	5	5	5	5

Individual Maternal Body Weight Gains (g)

Sex: Female — Body Weight Gain

10 mg/kg/day	Day(s) Relative to Mating (L)						
	13 → 14	14 → 15	15 → 16	16 → 17	17 → 18	18 → 19	19 → 20
301	1.1	7.2	7.3	19.9	7.1	15.9	12.6
302	-1.1	7.0	10.8	13.4	18.5	15.2	14.1
303	9.8	5.6	10.8	19.7	14.0	16.1	10.5
304	4.1	1.8	9.5	9.7	20.4	14.3	13.9
305	-2.7	14.8	5.7	7.0	21.6	14.5	8.9
Mean	2.2	7.3	8.8	13.9	16.3	15.2	12.0
SD	4.9	4.7	2.3	5.8	5.9	0.8	2.2
N	5	5	5	5	5	5	5

Sex: Female — Body Weight Gain

100 mg/kg/day	Day(s) Relative to Mating (L)						
	13 → 14	14 → 15	15 → 16	16 → 17	17 → 18	18 → 19	19 → 20
401	6.9	5.0	7.7	14.5	21.5	11.3	8.7
402	2.3	11.8	4.1	20.0	14.5	19.6	9.0
403	0.7	9.4	16.8	4.5	16.7	17.6	10.6
404	0.1	9.7	9.4	16.6	17.5	9.9	18.3
405	5.1	6.4	11.5	14.2	19.6	12.0	15.2
Mean	3.0	8.5	9.9	14.0	18.0	14.1	12.4
SD	2.9	2.7	4.7	5.8	2.7	4.3	4.2
N	5	5	5	5	5	5	5

Individual Maternal Body Weight Gains (g)

Sex: Female — Body Weight Gain

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	13 → 14	14 → 15	15 → 16	16 → 17	17 → 18	18 → 19	19 → 20
501	10.3	7.5	9.4	19.6	20.3	18.6	5.4
502	0.3	10.5	7.3	16.7	25.7	6.5	8.8
503	9.0	6.5	8.0	21.5	13.6	4.8	-3.9
504	1.6	10.2	12.6	16.4	21.1	10.3	10.4
505	8.9	2.1	5.5	17.9	13.9	18.0	0.2
Mean	6.0	7.4	8.6	18.4	18.9	11.6	4.2
SD	4.7	3.4	2.7	2.1	5.2	6.4	6.0
N	5	5	5	5	5	5	5

Sex: Female — Body Weight Gain

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	13 → 14	14 → 15	15 → 16	16 → 17	17 → 18	18 → 19	19 → 20
601	6.1	8.6	13.3	12.2	18.6	19.4	1.6
602	7.1	-2.5	11.8	7.1	17.9	14.2	2.8
603	5.8	4.7	5.6	11.7	17.7	12.8	-4.0
604	7.5	7.0	11.0	15.3	25.0	11.8	15.6
605	-0.2	11.9	8.0	17.0	15.3	18.5	5.6
Mean	5.3	5.9	9.9	12.7	18.9	15.3	4.3
SD	3.1	5.4	3.1	3.8	3.6	3.4	7.2
N	5	5	5	5	5	5	5

Appendix G
Individual Maternal Food Consumption

Individual Maternal Food Consumption (g/animal/day)

Sex: Female

Food Consumption Mean

0 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 8	8 → 10	10 → 12	12 → 14	14 → 16	16 → 18	18 → 20
101	18.7	17.8	19.1	21.2	27.5	24.0	20.4
102	23.8	19.9	23.1	24.6	22.7	28.2	24.0
103	25.7	27.5	29.2	32.0	28.3	33.2	30.2
104	22.0	18.9	18.1	24.9	25.2	28.2	26.6
105	16.2	16.1	23.0	21.1	20.7	25.1	25.5

Sex: Female

Food Consumption Mean

5 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 8	8 → 10	10 → 12	12 → 14	14 → 16	16 → 18	18 → 20
201	18.6	19.1	22.5	20.5	22.5	23.1	22.8
202	21.9	20.7	24.6	23.4	22.6	25.6	25.8
203	19.4	20.1	23.4	22.2	21.4	27.4	25.1
204	20.6	23.2	24.3	21.8	23.6	27.1	24.3
205	22.2	22.7	24.8	24.2	21.8	10.2	22.1

Sex: Female

Food Consumption Mean

10 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 8	8 → 10	10 → 12	12 → 14	14 → 16	16 → 18	18 → 20
301	23.4	24.6	26.1	26.5	26.0	27.7	24.9
302	16.0	22.2	25.4	21.7	20.8	23.8	23.6
303	23.7	24.3	30.8	30.7	29.5	33.8	31.4
304	13.4	18.9	22.7	21.4	21.9	26.7	28.0
305	18.0	17.0	21.6	21.5	21.6	24.9	24.1

Individual Maternal Food Consumption (g/animal/day)

Sex: Female

Food Consumption Mean

100 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 8	8 → 10	10 → 12	12 → 14	14 → 16	16 → 18	18 → 20
401	25.2	21.8	25.9	25.0	25.7	26.3	27.4
402	24.2	27.1	26.5	27.2	26.9	29.7	29.2
403	23.0	26.9	28.2	26.1	28.9	28.2	29.1
404	22.8	22.4	24.2	25.1	23.8	29.0	25.6
405	21.6	20.4	24.2	26.4	27.0	26.8	24.4

Sex: Female

Food Consumption Mean

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 8	8 → 10	10 → 12	12 → 14	14 → 16	16 → 18	18 → 20
501	21.1	20.8	20.7	24.1	26.1	27.6	27.6
502	15.5	16.1	19.1	20.9	23.4	25.6	23.7
503	18.2	18.3	24.3	28.7	26.9	31.6	20.7
504	16.8	13.9	22.3	25.1	26.5	30.3	29.3
505	13.8	22.2	26.9	26.4	24.8	26.0	24.5

Sex: Female

Food Consumption Mean

1000 mg/kg/day	Day(s) Relative to Mating (L)						
	6 → 8	8 → 10	10 → 12	12 → 14	14 → 16	16 → 18	18 → 20
601	16.9	19.0	22.5	24.3	25.2	26.4	26.6
602	11.9	14.9	17.0	19.0	16.4	18.4	20.2
603	18.5	21.2	23.0	22.2	23.3	24.4	22.6
604	11.2	15.7	16.8	21.6	23.0	26.4	25.1
605	15.2	20.2	18.3	19.9	24.8	26.1	26.1

Appendix H
Individual Maternal Gross Observations

Individual Maternal Gross Observations

Group: 1 Dose: 0 mg/kg/day Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
101	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
102	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
103	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
104	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
105	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions

Group: 2 Dose: 5 mg/kg/day Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
201	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
202	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
203	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
204	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
205	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions

Individual Maternal Gross Observations

Group: 3 Dose: 10 mg/kg/day Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
301	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
302	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
303	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
304	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
305	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions

Group: 4 Dose: 100 mg/kg/day Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
401	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
402	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
403	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
404	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
405	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions

Individual Maternal Gross Observations

Group: 5 Dose: 1000 mg/kg/day Sex: Female

Animal Ref.	Mode Of Death	Death Day	Death (Week)	Observation(s)
501	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
502	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
503	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
504	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
505	SCHEDULED SACRIFICE	20	(2)	LIVER (2); left; Discoloration; tan; 0.5 cm or less >0.5 Any remaining protocol required tissues, which have been examined, have no visible lesions

Group: 6 Dose: 1000 mg/kg/day Sex: Female

Animal Ref.	Mode Of Death	Death Day	Death (Week)	Observation(s)
601	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
602	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
603	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
604	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions
605	SCHEDULED SACRIFICE	20	(2)	No Visible Lesions

Appendix I
Individual Reproductive Data

INDIVIDUAL REPRODUCTIVE DATA

EXPLANATORY NOTES

ABBREVIATIONS:

% / Mean	-	percentage or mean of the individual values for the group
A	-	abortion
E	-	excluded
N	-	number of values used in calculation
NP	-	not pregnant
S.D.	-	standard deviation
TLL	-	total litter loss
U	-	unscheduled death

NOTES:

All A, E, NP, TLL, and U dams are excluded from all group summary calculations.

A column showing late resorptions appears in this appendix only if at least one late resorption occurred in the study.

Indices, which compare Implantations with *Corpora Lutea*, do not include animals, with an Implantation count > *Corpora Lutea* Count.

Indices, which compare Foetuses with Implantations, do not include animals, with a Foetus count > Implantation Count.

Individual Reproductive Data

Group: 1 0 mg/kg/day

Dam Number	Number of Implantations Left	Right	- Number of Intra-Uterine Deaths - Early Resorption L	R	Total Deaths L	R	% Post-implantation Loss	Number of live foetuses Left	Right	Live Foetuses as % of implantations
101	10	3	0	0	0	0	0.0	10	3	100.0
102	1	7	0	0	0	0	0.0	1	7	100.0
103	5	6	0	0	0	0	0.0	5	6	100.0
104	7	5	0	0	0	0	0.0	7	5	100.00
105	5	8	0	0	0	0	0.0	5	8	100.0
	21	24	0	0	0	0		21	24	
TOTAL	45		0		0			45		
Litter Mean	11.3						0.0	11.3		100.0
% / Mean							0.0			100.0
S.D.	2.4							2.4		
N	4		4		4			4		

Group: 2 5 mg/kg/day

Dam Number	Number of Implantations Left	Right	- Number of Intra-Uterine Deaths - Early Resorption L	R	Total Deaths L	R	% Post-implantation Loss	Number of live foetuses Left	Right	Live Foetuses as % of implantations
201	7	4	0	0	0	0	0.0	7	4	100.0
202	9	6	0	0	0	0	0.0	9	6	100.0
203	6	7	0	0	0	0	0.0	6	7	100.0
204	7	7	0	0	0	0	0.0	7	7	100.0
205	3	8	0	0	0	0	0.0	3	8	100.0
	32	32	0	0	0	0		32	32	
TOTAL	64		0		0			64		
Litter Mean	12.8						0.0	12.8		100.0
% / Mean							0.0			100.0
S.D.	1.8							1.8		
N	5		5		5			5		

Individual Reproductive Data

Group: 3 10 mg/kg/day

Dam Number	Number of Implantations Left	Right	- Number of Intra-Uterine Deaths - Early Resorption L	R	Total Deaths L	R	% Post-implantation Loss	Number of live foetuses Left	Right	Live Foetuses as % of implantations
301	6	7	0	0	0	0	0.0	6	7	100.0
302	9	6	0	0	0	0	0.0	9	6	100.0
303	8	5	0	0	0	0	0.0	8	5	100.0
304	5	6	0	0	0	0	0.0	5	6	100.0
305	4	7	1	0	1	0	9.1	3	7	90.9
	32	31	1	0	1	0		31	31	
TOTAL	63		1		1			62		
Litter Mean	12.6		0.2		0.2		1.8	12.4		98.2
% / Mean							1.6			98.4
S.D.	1.7		0.4		0.4			1.9		
N	5		5		5			5		

Group: 4 100 mg/kg/day

Dam Number	Number of Implantations Left	Right	- Number of Intra-Uterine Deaths - Early Resorption L	R	Total Deaths L	R	% Post-implantation Loss	Number of live foetuses Left	Right	Live Foetuses as % of implantations
401	7	5	0	0	0	0	0.0	7	5	100.0
402	5	8	0	0	0	0	0.0	5	8	100.0
403	6	5	0	0	0	0	0.0	6	5	100.0
404	5	8	0	0	0	0	0.0	5	8	100.0
405	6	9	0	0	0	0	0.0	6	9	100.0
	29	35	0	0	0	0		29	35	
TOTAL	64		0		0			64		
Litter Mean	12.8						0.0	12.8		100.0
% / Mean							0.0			100.0
S.D.	1.5							1.5		
N	5		5		5			5		

Individual Reproductive Data

Group: 5 1000 mg/kg/day

Dam Number	Number of Implantations Left	Right	- Number of Intra-Uterine Deaths - Early Resorption L	R	Total Deaths L	R	% Post-implantation Loss	Number of live foetuses Left	Right	Live Foetuses as % of implantations
501	8	5	0	0	0	0	0.0	8	5	100.0
502	5	7	0	0	0	0	0.0	5	7	100.0
503	9	6	0	0	0	0	0.0	9	6	100.0
504	4	8	0	0	0	0	0.0	4	8	100.0
505	6	7	0	0	0	0	0.0	6	7	100.0
	32	33	0	0	0	0		32	33	
TOTAL	65		0		0			65		
Litter Mean	13.0						0.0	13.0		100.0
% / Mean							0.0			100.0
S.D.	1.2							1.2		
N	5		5		5			5		

Group: 6 1000 mg/kg/day

Dam Number	Number of Implantations Left	Right	- Number of Intra-Uterine Deaths - Early Resorption L	R	Total Deaths L	R	% Post-implantation Loss	Number of live foetuses Left	Right	Live Foetuses as % of implantations
601	5	8	0	0	0	0	0.0	5	8	100.0
602	6	5	0	0	0	0	0.0	6	5	100.0
603	3	13	0	0	0	0	0.0	3	13	100.0
604	5	8	0	0	0	0	0.0	5	8	100.0
605	5	8	0	0	0	0	0.0	5	8	100.0
	24	42	0	0	0	0		24	42	
TOTAL	66		0		0			66		
Litter Mean	13.2						0.0	13.2		100.0
% / Mean							0.0			100.0
S.D.	1.8							1.8		
N	5		5		5			5		

Appendix J
Individual Animal Organ Weight Data

INDIVIDUAL ANIMAL ORGAN WEIGHTS

KEY TO APPENDIX

CALCULATED MEASUREMENT FORMULAE:

Organ/Body Weight = Organ Weight (g)/Body Weight (g) x 100

ABBREVIATIONS:

g - gram

NOTES:

Due to rounding differences, values in tables may be slightly different than appendices.

Individual Animal Organ Weight Data

0 mg/kg/day	Kidneys Weight (g)	Kidney /Terminal Bodywei	Liver Weight (g)	Liver /Terminal Bodyweig
101	1.82	0.496	13.40	3.652
102	1.77	0.504	14.78	4.206
103	2.33	0.549	17.96	4.234
104	1.82	0.470	15.27	3.947
105	2.11	0.573	16.18	4.392
N	5	5	5	5

5 mg/kg/day	Kidneys Weight (g)	Kidney /Terminal Bodywei	Liver Weight (g)	Liver /Terminal Bodyweig
201	1.88	0.526	14.14	3.955
202	1.92	0.501	16.90	4.406
203	2.20	0.569	16.27	4.207
204	2.06	0.520	15.00	3.787
205	1.87	0.520	14.94	4.151
N	5	5	5	5

10 mg/kg/day	Kidneys Weight (g)	Kidney /Terminal Bodywei	Liver Weight (g)	Liver /Terminal Bodyweig
301	2.18	0.563	15.70	4.056
302	1.95	0.510	15.27	3.997
303	2.11	0.503	17.38	4.143
304	2.06	0.593	14.36	4.131
305	1.86	0.527	15.12	4.283
N	5	5	5	5

Individual Animal Organ Weight Data

100 mg/kg/day	Kidneys Weight (g)	Kidney /Terminal Bodywei	Liver Weight (g)	Liver /Terminal Bodyweig
401	2.01	0.530	16.29	4.294
402	2.29	0.575	18.14	4.555
403	2.31	0.562	18.39	4.471
404	2.02	0.507	18.31	4.596
405	2.18	0.545	15.84	3.961
N	5	5	5	5

1000 mg/kg/day	Kidneys Weight (g)	Kidney /Terminal Bodywei	Liver Weight (g)	Liver /Terminal Bodyweig
501	1.95	0.510	17.65	4.619
502	1.94	0.544	18.83	5.282
503	2.13	0.564	17.12	4.536
504	2.20	0.611	17.98	4.990
505	2.31	0.605	19.79	5.186
N	5	5	5	5

1000 mg/kg/day	Kidneys Weight (g)	Kidney /Terminal Bodywei	Liver Weight (g)	Liver /Terminal Bodyweig
601	2.17	0.589	17.90	4.860
602	1.89	0.578	16.50	5.044
603	1.90	0.512	17.89	4.817
604	2.09	0.565	20.02	5.408
605	2.18	0.583	18.01	4.819
N	5	5	5	5

Appendix K
Quantitation of Plasma Samples for H-28548: Toxicokinetic Study in Pregnant Rats

TRADE SECRET

STUDY TITLE: Quantitation of Plasma Samples for H-28548: Toxicokinetic Study in Pregnant Rats

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WORK REQUEST NUMBER: 18405

SERVICE CODE NUMBER: 849

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SUMMARY

The dose response curve was linear between 5 and 100 mg/kg/day. At 1000 mg/kg/day the concentration was less than what would be predicted if the dose response curve was linear through 1000 mg/kg/day.

The mean plasma concentration on day 20 was less than the mean plasma concentration on day 6. This implies that steady state was achieved by day 6 and that there is no accumulation in the dams between day 6 and day 20.

The concentration in plasma pooled from pups was approximately one-third of the concentration in plasma from the dam at the same time point.

MATERIALS AND METHODS

A. Sample Preparation and Chemical Analysis

1. Sample Receipt

The plasma samples were received and stored frozen upon laboratory receipt, and when not in use. The plasma samples consisted of the following groupings.

Sample Description	Group	Dosage (mg/kg/day)[a]	Gestation Day	Number of Samples
Rat Plasma, Female	6	1000	6	5
Rat Plasma, Female	1	0	20	5
Rat Plasma, Female	2	5	20	5
Rat Plasma, Female	3	10	20	5
Rat Plasma, Female	4	100	20	5
Rat Plasma, Female	5	1000	20	5
Rat Plasma, Female	6	1000	20	5
Rat Plasma, Pooled Fetus Litter	1	0	20	5
Rat Plasma, Pooled Fetus Litter	2	5	20	5
Rat Plasma, Pooled Fetus Litter	3	10	20	5
Rat Plasma, Pooled Fetus Litter	4	100	20	5
Rat Plasma, Pooled Fetus Litter	5	1000	20	5
Rat Plasma, Pooled Fetus Litter	6	1000	20	5

a Adjusted for purity

2. Sample Preparation Procedure

The frozen plasma study samples were thawed to room temperature and mixed briefly. A pipette was used to transfer 50 µL of plasma sample into a 1.7 mL microcentrifuge tubes. The pipette was then used to add 50 µL of internal standard. The internal standard contained perfluoro-n-(1,2 $^{13}C_2$) hexanoic acid (MPFHxA) at a concentration of 100 ng/mL in saline. The saline contained 0.9% (w/v) sodium chloride in HPLC grade water. The pipette was then used to add 300 µL acetonitrile to the tubes which were then vortexed to mix homogenously. The tubes were centrifuged at 14,000 RCF for 10 minutes at room temperature. A pipette was used to transfer 800 µL of HPLC grade water into HPLC vials, and 200 µL sample supernatant. The initial preparation factor was 40x. Additional sample dilutions were performed using an internal standard dilution solution, which contained MPFHx at a concentration of 2.5 ng/mL. The dilution solution solvent contained 15% acetonitrile in HPLC grade water. Quality control samples were also prepared in control rat plasma matrix at low, mid, and high fortification levels.

The prepared calibration standards, QC samples, and study samples were analyzed by LC/MS/MS to quantify the amount of H-28548 in the plasma samples.

3. Stock Solutions and Calibration Standards

A stock solution of H-28548 (purity 84.0%) was prepared in HPLC grade water. The stock solution was then used to prepare calibration standards at different levels in 15% acetonitrile in HPLC grade water solvent containing the MPFHxA internal standard at a concentration of 2.5 ng/mL.

B. Instrument and Conditions

The prepared plasma samples were quantified by high performance liquid chromatography with detection by tandem mass spectrometry (LC/MS/MS). The instrument was configured as follows:

HPLC Instrument:	Agilent Model 1100
MS Instrument:	Applied Biosystems MDS Sciex API 4000
Software:	Analyst version 1.4.2

LC Parameters:

Column:	Zorbax SB-C8 2.1x100mm with 3.5 micron particle size
Mobile Phase:	A: 0.15% acetic acid in HPLC Grade water B: 0.15% acetic acid in acetonitrile
Column Temperature:	35.0°C
Injection Volume:	75 µL

MS Parameters:

Ion Source:	Turbo Spray, Negative Ion
Valco Divert Valve:	0 min to waste, 2.3 min to source, 9 min to waste
Collision Gas:	Nitrogen

Temperature (TEM): 120
Dwell: 250 msec
Curtain Gas Flow (CUR): 10.0
GS1: 25.0
GS2: 25.0
IonSpray (IS) Voltage: -4500
CAD: 6.0

Quadrupole Resolution: Quad. 1: Unit
Quad. 3: Unit

MRM Settings:	Q1 Mass	Q3 Mass	DP	EP	CE	CXP
H-28548	329	285	-20	-10	-6	-7
MPFHx	315	270	-30	-10	-12	-11

HPLC Gradient:

Step	Total Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0	0.00	400	68.0	32.0
1	4.00	400	68.0	32.0
2	5.00	400	5.0	95.0
3	7.00	400	5.0	95.0
4	7.10	400	68.0	32.0
5	9.00	400	68.0	32.0

C. Quantitation

The calibration standard curve was generated by regression analysis using the chromatographic peak areas of the calibration standard solutions analyzed in duplicate. Data for test solutions were compared to the calibration standard curve to determine concentrations of H-28548. Additional sample dilutions were performed to ensure that the peak area responses were within the calibration curve.

RESULTS AND DISCUSSION

A. Calibration Standard Curve

(Figure 1)

A calibration curve for H-28548 is shown in Figure 1. The curve was generated based on the internal standard technique using a quadratic equation, and 1/x weighing.

B. Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined from the average peak-to-peak noise of duplicate control plasma samples versus the peak height response of the lowest calibration standard. The LOD was calculated as the concentration equivalent of a peak that has 3 times the noise response. The LOQ was based on the lowest calibration standard concentration, which had at reponse at least 10 times higher than the noise response. The overall method LOD and LOQ were calculated by multiplying by the plasma sample preparation factor of 40x. The LOD and LOQ for H-28548 in plasma was 0.7 ng/mL and 3.00 ng/mL, respectively.

C. Fortification QC Sample Results

(Table 1)

The average QC fortification recovery results for H-28548 in plasma are provided in Table 1. The average recoveries are within 95-106%, and demonstrate good method performance throughout the study sample analyses.

D. Plasma Sample Results

(Table 2, Figures 2-5, Appendix A)

Example chromatograms of a) dilution solvent blank, b) lowest calibration standard , c) QC control plasma sample, and d) plasma study sample from group 2 on GD 20 after an additional 50x sample dilution are shown in Figure 2. The H-28548 elutes as a well resolved peak at a retention time of approximately 3.2 minutes.

The individual plasma sample results are provided in Appendix A, and are summarized in Table 2.

The control samples had quantifiable levels of H-28548. The average trace level concentration of H-28548 in plasma from the control rats were 59x and 120x lower than the concentrations in the lowest dosed group 2 samples for the GD 20 pooled pups and dams, respectively.

The dose response curve was linear between 5 and 100 mg/kg/day. At 1000 mg/kg/day the concentration was less than what would be predicted if the dose response curve was linear through 1000 mg/kg/day. This could be due to either saturation of absorption or an increase in elimination rate at the highest dose level (Table 2 and Figure 3).

The mean plasma concentration on day 20 was less than the mean plasma concentration on day 6. This implies that steady state was achieved by day 6 and that there is no accumulation in the dams between day 6 and day 20 (Table 2 and Figure 4).

The concentration in plasma pooled from pups was approximately one-third of the concentration in plasma from the dam at the same time point. This was true at all dose levels except for 100 mg/kg/day where the pup:dam plasma ratio was slightly lower at 0.2 (Table 2 and Figure 5).

CONCLUSIONS

The dose response curve was linear between 5 and 100 mg/kg/day. At 1000 mg/kg/day the concentration was less than what would be predicted if the dose response curve was linear through 1000 mg/kg/day.

The mean plasma concentration on day 20 was less than the mean plasma concentration on day 6. This implies that steady state was achieved by day 6 and that there is no accumulation in the dams between day 6 and day 20.

The concentration in plasma pooled from pups was approximately one-third of the concentration in plasma from the dam at the same time point.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

TABLES

Table 1
Average QC Plasma Fortification Recoveries for H-28548

Fortification Level[a]	Fortification Concentration (ng/mL)	Average Recovery (%%	CV (%)
Low	6.00	95	2
Mid	1500	105	7
High	100,000	106	2

a n=6 replicates

Table 2
Summary of Plasma Concentrations for Parent Compound H-28548

		Concentration (ng/mL)						
		Dams				Pooled Pups		
	Dose	Day 6		Day 20		Day 20		Pup:Dam
Group	(mg/kg/day)	Mean	SD	Mean	SD	Mean	SD	Plasma Ratio
1	0			33	16	19	23	
2	5			3984	469	1134	175	0.3
3	10			9312	1710	2458	465	0.3
4	100			85560	10092	18320	9128	0.2
5	1000			338400	160168	99800	26482	0.3
6	1000	430600	162712	348400	130362	102240	28295	0.3

FIGURES

Figure 1
Calibration Curves for H-28548

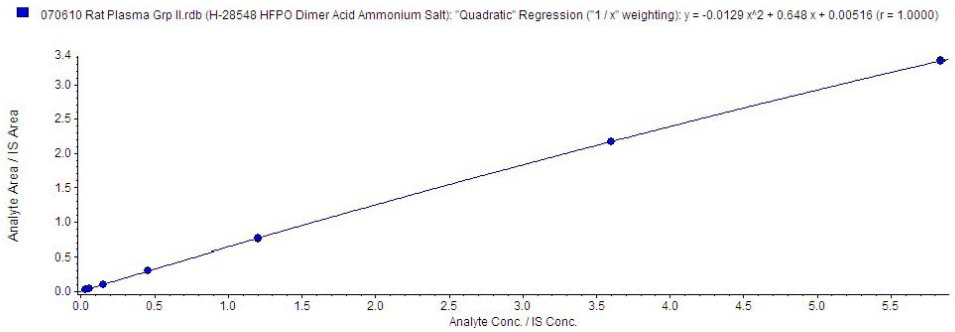
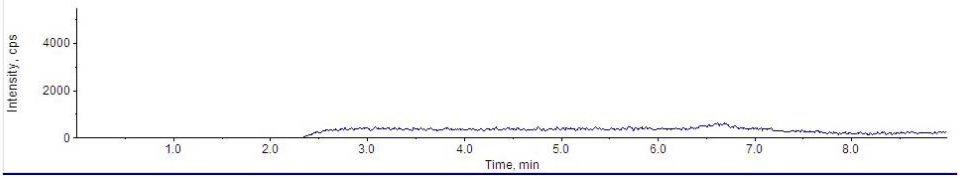
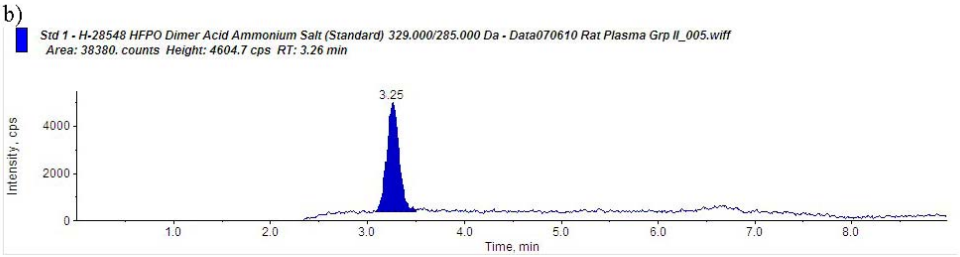


Figure 2
Chromatograms for H-28548 of a) dilution solvent blank, b) Lowest Calibration Standard, c) QC Control Plasma Sample, and d) Group 2 Day 20 Plasma After an Additional 50x Sample Dilution

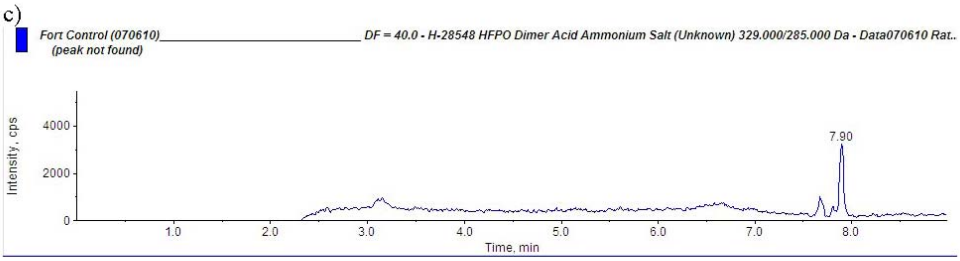
a)



b)



c)



d)

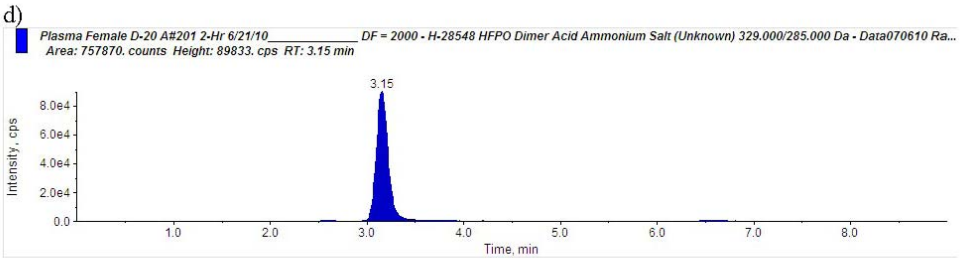


Figure 3
Dose response curve for H-28548

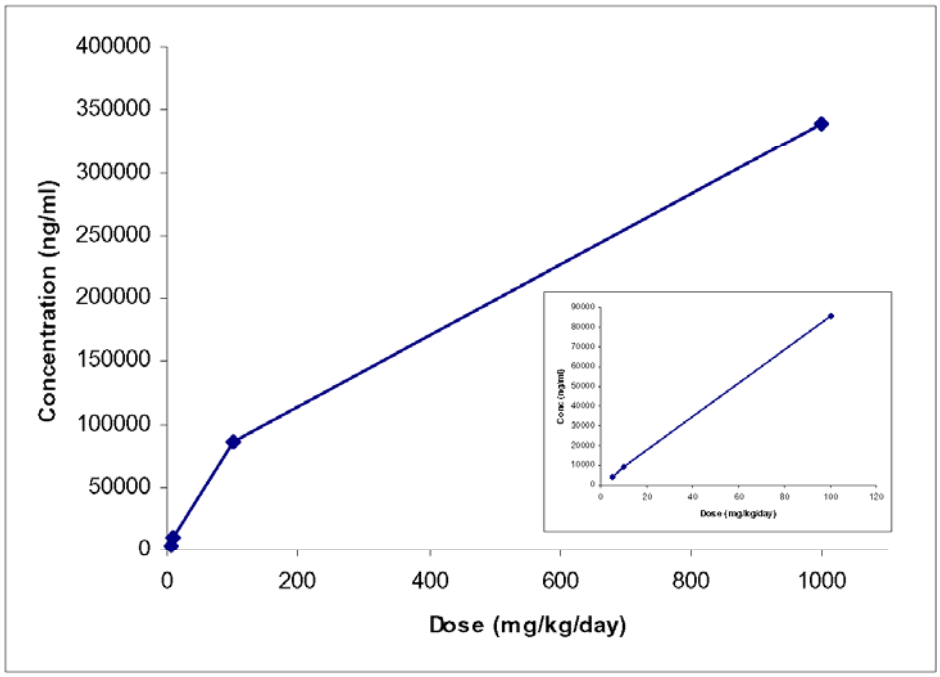


Figure 4
Plasma Concentration of H-28548 on Day 6 and Day 20 in Dams After 1000 mg/kg/day Dose

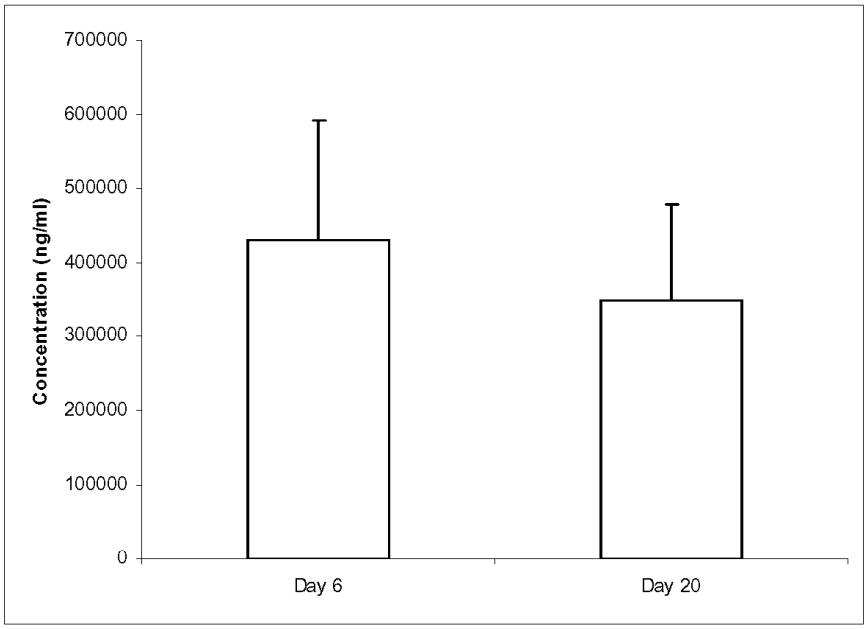
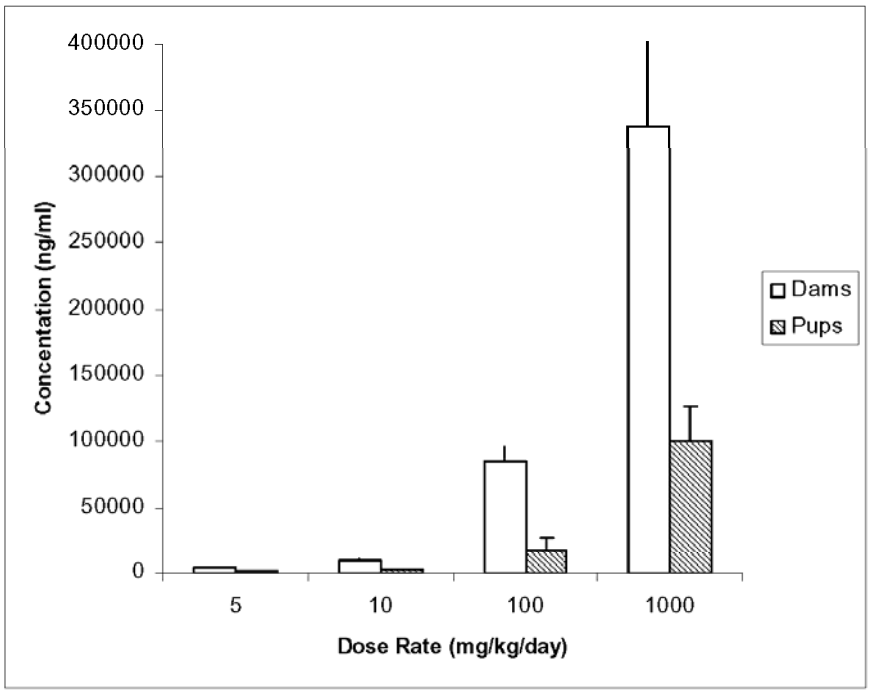


Figure 5
Plasma Concentration of H-28548 on Day 20 in Dams and Pups



APPENDICES

Appendix A
Individual Plasma Concentrations of H-28548

Individual Plasma Concentrations of H-28548

Sample Name	Group	Dosage (mg/kg/day)	H-28548 (ng/mL)
Plasma Female D-20 A#101 2-Hr 6/21/10	1	0	54.7
Plasma Female D-20 A#102 2-Hr 6/21/10	1	0	20.6
Plasma Female D-20 A#103 2-Hr 6/21/10	1	0	19.1
Plasma Female D-20 A#104 2-Hr 6/21/10	1	0	45.2
Plasma Female D-20 A#105 2-Hr 6/21/10	1	0	26.3
Plasma Female D-20 A#201 2-Hr 6/21/10	2	5	3800
Plasma Female D-20 A#202 2-Hr 6/21/10	2	5	4640
Plasma Female D-20 A#203 2-Hr 6/21/10	2	5	3530
Plasma Female D-20 A#204 2-Hr 6/21/10	2	5	3650
Plasma Female D-20 A#205 2-Hr 6/21/10	2	5	4300
Plasma Female D-20 A#301 2-Hr 6/21/10	3	10	6640
Plasma Female D-20 A#302 2-Hr 6/21/10	3	10	8940
Plasma Female D-20 A#303 2-Hr 6/21/10	3	10	9580
Plasma Female D-20 A#304 2-Hr 6/21/10	3	10	11200
Plasma Female D-20 A#305 2-Hr 6/21/10	3	10	10200
Plasma Female D-20 A#401 2-Hr 6/21/10	4	100	98700
Plasma Female D-20 A#402 2-Hr 6/21/10	4	100	88300
Plasma Female D-20 A#403 2-Hr 6/21/10	4	100	85400
Plasma Female D-20 A#404 2-Hr 6/21/10	4	100	84900
Plasma Female D-20 A#405 2-Hr 6/21/10	4	100	70500
Plasma Female D-20 A#501 2-Hr 6/21/10	5	1000	309000
Plasma Female D-20 A#502 2-Hr 6/21/10	5	1000	269000
Plasma Female D-20 A#503 2-Hr 6/21/10	5	1000	621000
Plasma Female D-20 A#504 2-Hr 6/21/10	5	1000	237000
Plasma Female D-20 A#505 2-Hr 6/21/10	5	1000	256000
Plasma Female D-20 A#601 2-Hr 6/21/10	6	1000	194000
Plasma Female D-20 A#602 2-Hr 6/21/10	6	1000	459000
Plasma Female D-20 A#603 2-Hr 6/21/10	6	1000	300000
Plasma Female D-20 A#604 2-Hr 6/21/10	6	1000	507000
Plasma Female D-20 A#605 2-Hr 6/21/10	6	1000	282000
Plasma Day-6 2-Hr A#601 6/7/10	6	1000	343000
Plasma Day-6 2-Hr A#602 6/7/10	6	1000	378000
Plasma Day-6 2-Hr A#603 6/7/10	6	1000	720000
Plasma Day-6 2-Hr A#604 6/7/10	6	1000	339000
Plasma Day-6 2-Hr A#605 6/7/10	6	1000	373000
Plasma Dams Pooled Litter D-20 A#101 6/21/10	1	0	6.59
Plasma Dams Pooled Litter D-20 A#102 6/21/10	1	0	58.2
Plasma Dams Pooled Litter D-20 A#103 6/21/10	1	0	5.73
Plasma Dams Pooled Litter D-20 A#104 6/21/10	1	0	4.41
Plasma Dams Pooled Litter D-20 A#105 6/21/10	1	0	20.1
Plasma Dams Pooled Litter D-20 A#201 6/21/10	2	5	1100
Plasma Dams Pooled Litter D-20 A#202 6/21/10	2	5	1020
Plasma Dams Pooled Litter D-20 A#203 6/21/10	2	5	989
Plasma Dams Pooled Litter D-20 A#204 6/21/10	2	5	1430
Plasma Dams Pooled Litter D-20 A#205 6/21/10	2	5	1130
Plasma Dams Pooled Litter D-20 A#301 6/21/10	3	10	2020
Plasma Dams Pooled Litter D-20 A#302 6/21/10	3	10	2710
Plasma Dams Pooled Litter D-20 A#303 6/21/10	3	10	2030
Plasma Dams Pooled Litter D-20 A#304 6/21/10	3	10	3110
Plasma Dams Pooled Litter D-20 A#305 6/21/10	3	10	2420

Individual Plasma Concentrations of H-28548

Sample Name		Group	Dosage (mg/kg/day)	H-28548 (ng/mL)
Plasma Dams Pooled Litter D-20 A#401	6/21/10	4	100	34400
Plasma Dams Pooled Litter D-20 A#402	6/21/10	4	100	14800
Plasma Dams Pooled Litter D-20 A#403	6/21/10	4	100	14700
Plasma Dams Pooled Litter D-20 A#404	6/21/10	4	100	11700
Plasma Dams Pooled Litter D-20 A#405	6/21/10	4	100	16000
Plasma Dams Pooled Litter D-20 A#501	6/21/10	5	1000	97200
Plasma Dams Pooled Litter D-20 A#502	6/21/10	5	1000	106000
Plasma Dams Pooled Litter D-20 A#503	6/21/10	5	1000	135000
Plasma Dams Pooled Litter D-20 A#504	6/21/10	5	1000	100000
Plasma Dams Pooled Litter D-20 A#505	6/21/10	5	1000	60800
Plasma Dams Pooled Litter D-20 A#601	6/21/10	6	1000	70600
Plasma Dams Pooled Litter D-20 A#602	6/21/10	6	1000	134000
Plasma Dams Pooled Litter D-20 A#603	6/21/10	6	1000	121000
Plasma Dams Pooled Litter D-20 A#604	6/21/10	6	1000	111000
Plasma Dams Pooled Litter D-20 A#605	6/21/10	6	1000	74600